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CHROMOSOMES OF NINE SPECIES OF SCHISTOSOMES*

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Since Price (1929) published his synopsis of the family Schistosomatidae, numerous species of adult worms have been described and new genera and subfamilies have been proposed. In some instances relationships of species and genera seem uncertain and the bases of some genera appear questionable. A number of life cycles have been elucidated, but there still remain many species of adult worms with unsolved life cycles, and cercariae have been described without knowledge of adult stages. It was thought that cytological studies might help in solving some of the taxonomic problems in this group and eventually might furnish clues as to the origin of the dioecious schistosomes from hermaphroditic ancestors.

The literature contains only a few reports on chromosomes of schistosomes. Earlier works on *Schistosoma haematobium* by Lindner (1914) and on *S. japonicum* by Faust and Meleney (1924) and Severinghaus (1928) reported a difference in chromosome number between the sexes with the male interpreted as being heterogametic. Later studies, however, on *S. japonicum* by Ikeda and Makino (1936) and on *S. mansoni* by Niyamasena (1940) indicated neither a difference in chromosome number nor morphologically distinguishable sex chromosomes. Other more or less incidental observations on *S. mansoni* include Vogel's (1942) report of 16 chromosomes in male and female material from snail infections (probably of parthenogenetic origin) and Gönnert's (1955) finding of 8 bivalents in secondary spermatocytes. Recent investigations in this laboratory have shown that in *Schistosomatium douthitti* there are clearly distinguishable sex chromosomes and the female is heterogametic (Short, 1957; Short and Menzel, 1959a). Studies by Walton (1959) substantiate these results on *S. douthitti.*

The present paper deals with chromosomes in cercarial embryos of 9 species of schistosomes in 6 genera. Abstracts have been published on certain aspects of this work (Short and Menzel, 1957, 1959b).

Table I lists the species with snail hosts and origin of schistosome material. The first 4 species are mammalian parasites. The 3 species of *Schistosoma* are familiar parasites of man. *Schistosomatium douthitti* also is a rather well known schistosome which occurs in the northern United States (see Kagan et al, 1954). The cercaria of *S. douthitti* was described by Cort (1914, 1915) and its life cycle elucidated by Price (1931). An idiogram for this species is presented here for the first time and the chromosomes are pictured for comparison with those of the

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other species studied.

The last five schistosomes of table I are avian parasites. *Ornithobilharzia*

TABLE I. *Chromosomes in cercarial embryos of schistosomes.*

Species	Source	Snail species	Sex snail	No. snails	No. plates	Mitotic chromosomes (2n)
Schistosomatium douthitti (Cort, 1914) Price, 1931*	Chicago, Illinois	*Lymnaea palustris*	male	24	198	14, XXAA
			"	1	5	21, XXYAAA
			female	24	238	14, XYAA
			"	1	7	21, XYYAAA
Schistosoma mansoni Sambon, 1907	Puerto Rico	*Australorbis glabratus*	male	11	87	16
			female	8	63	16
	Egypt	"	male	5	30	16
			female	3	26	16
	Liberia	"	male	1	2	16
			female	2	24	16
Schistosoma haematobium (Bilharz, 1852) Weinland, 1858	West Africa	*Bulinus globosus*	male	1	6	16
			female	4	46	16
			?	4	28	16
Schistosoma japonicum Katsurada, 1904	Philippine Islands	*Oncomelania quadrasi*	?	7	39	16
Ornithobilharzia canaliculata (Rudolphi, 1819) Odhner, 1912	Tampa Bay, Florida	*Bctillaria minima*	male	3	61	16, XXAA
			male?	6	35	16, XXAA
			female	3	60	16, XYAA
			female?	8	95	16, XYAA
			mixed?	11	107	16, XXAA & XYAA
Trichobilharzia physellae (Talbot, 1936) McMullen and Beaver, 1945	Douglas Lake, Michigan	*Physa parkeri, P. magnalacustris, Physa* sp.	?	11	112	16
Trichobilharzia stagnicolae (Talbot, 1936) McMullen and Beaver, 1945, A	"	*Stagnicola emarginata angulata*	?	2**	27	16
T. stagnicolae, B	"	"	?	4**	31	18
Austrobilharzia variglandis (Miller and Northup, 1926) Penner, 1953	Portland Harbor, Maine	*Nassarius obsoletus*	male†	1	57	16
Gigantobilharzia huronensis Najim, 1950	Near Ann Arbor, Michigan	*Physa* sp.	female?††	1	9	16, XYAA?
			male?		15	16, XXAA?

* These data on *S. douthitti* have been published (Short, 1957).
** One snail harbored cercarial embryos with 16 and 18 chromosomes.
† Only 2 male worms were recovered from 1 parakeet.
†† Twenty-six female worms were recovered from 3 chicks.

canaliculata was first reported by Rudolphi (1819) as *Distoma canaliculatum* from *Sterna* sp. from Brazil. Adults of this fluke have been recorded subsequently from Brazil (Braun, 1902; Travassos, 1942) and both adults and cercariae from Florida (Penner, 1953a).

Cercariae of *Trichobilharzia physellae* and *T. stagnicolae* were described from the Douglas Lake region, Michigan, by Talbot (1936). Adult *T. physellae* were reported from natural infections by McLeod (1937) and McLeod and Little (1942); both species were recovered from experimentally infected birds by McMullen and Beaver (1945).

The cercaria of *Austrobilharzia variglandis* was described by Miller and Northup (1926) from the Massachusetts coast. Subsequent studies on life cycle, morphology, and hosts have been reported by Stunkard and Hinchliffe (1951, 1952), Penner (1953b), Chu and Cutress (1954), and Grodhaus and Keh (1958).

Gigantobilharzia huronensis was described and its life cycle worked out by Najim (1950, 1956) from material collected near Ann Arbor, Michigan.

MATERIALS AND METHODS

Chromosomes were analyzed in cercarial embryos of all species studied, and also in adult worms of *O. canaliculata*.

For study of chromosomes in cercarial embryos digestive glands of infected snails were fixed in fresh Carnoy's solution (1 part glacial acetic; 3 parts 95 percent ethanol) and stored in a freezer. Temporary iron-acetocarmine squashes of small pieces of digestive gland containing sporocysts with embryos gave satisfactory and often excellent preparations, which were, however, difficult to make permanent.

For chromosomes in adult worms, it was found that staining with Gomori's chrom-alum hematoxylin, according to a schedule suggested by Dr. Charles Ray, Jr. of Emory University, gave more uniformly good preparations which were easily made permanent by the method of Conger and Fairchild (1953). Mitotic chromosomes of adult male and female worms of *O. canaliculata* were studied in such preparations. This method also gave excellent permanent squash preparations of cercarial embryos. Some of the cells analyzed for *T. stagnicolae* and *O. canaliculata* were on such slides. The remainder of the mitotic figures reported, including all from which measurements were taken for idiograms, were from temporary acetocarmine squashes, since (1) for purposes of comparison a uniform method was essential and the Gomori stain was adopted after material of some species had been exhausted, and (2) although fixation was the same in both methods, the chromosomes were considerably smaller in haematoxylin than in acetocarmine squashes (compare figs. 9 and 12 with figs. 17 to 20).

In each species studied, individual chromosomes could all be identified in many of the best plates. For construction of idiograms, camera lucida drawings were made of such plates at a magnification of 5100×. Chromosome arms were then measured on the drawings to the nearest millimeter and the idiograms constructed to the scale of 1 mm equal to one-eighth inch. Table II

TABLE II. *Source of idiograms.*

Schistosome	No. snails	Sex of infection	No. plates
Schistosomatium douthitti	1	female	10 + 10 XY
Schistosoma mansoni (Puerto Rico)	1	male	10
S. mansoni (Egypt)	1	male	1
	2	female	9
S. mansoni (Liberia)	1	?	1
	2	female	9
S. haematobium	2	?	8
	1	female	2
S. japonicum	5	?	10
Ornithobilharzia canaliculata	1	female	10 + 10 XY
Trichobilharzia physellae	2	?	10
T. stagnicolae A	1	?	10
T. stagnicolae B	2	?	10
Austrobilharzia variglandis	1	male?	10
Gigantobilharzia huronensis	1	mixed?	6

presents information on the cells used. All idiograms are based on average arm-lengths of 20 chromosomes of each type from 10 complete plates, with the following exceptions: (1) In the idiogram of *T. stagnicolae* B, chromosome 8 is based on only 18 chromosomes; in the remaining two, the positions of the centromeres were uncertain. (2) In *S. douthitti* and *O. canaliculata*, for which both X and Y are shown, the X and Y are based on the 10 pairs in the same plates used for the autosomes plus an additional 10 XY pairs from other plates. (3) In the single snail infected with *G. huronensis*, only 6 completely analyzable cells were found. Chromosomes 2 to 8 each represent average arm lengths of 12 chromosomes. Three of the cells appeared homomorphic and 3 heteromorphic for chromosome 1, suggesting a mixed infection of XX and XY karyotypes similar to those of *O. canaliculata*. The diagram of chromosome 1 is based on the 9 long metacentric chromosomes; the questionable "Y" is omitted.

The average total lengths of the haploid chromosome sets were calculated and are shown in microns with the idiograms. Lengths of Y chromosomes are excluded from the totals.

OBSERVATIONS

Table I summarizes for each species the number of infected snails used, the sex of infection if known, the number of plates analyzed, and the somatic chromosome composition. Chromosome complements are pictured in plates I, II, and III, and are represented diagrammatically in the idiograms of plate IV.

The following reservations should be made in interpreting the idiograms: (1) In chromosomes with nearly median centromeres in which there was no criterion other than length for distinguishing the 2 arms, the shorter arm was always

taken as the "left" or upper arm in the idiogram. This may prejudice the diagrams slightly in the direction of inequality of arms for such chromosomes. (2) In some species, the satellite on chromosome 6 was very small and could not be seen in some cells. In such plates, the shorter pair of acrocentrics was taken as chromosome 6. The idiograms may therefore slightly exaggerate the differences in length of 5 and 6. (3) In the case of the smallest chromosomes, 7, 8, and 9, the exact position of the centromeres was often difficult to determine. The smallest chromosome of *S. douthitti* has a median centromere; the 2 smallest pairs of *O. canaliculata, A. variglandis* and the 3 species of *Schistosoma* consist of 1 acrocentric and 1 metacentric pair. The arm-length ratios for the other species may be slightly inaccurate because of errors in locating the centromeres and in matching homologues.

No attempt was made to indicate relative widths of chromomes in the idiograms because of the difficulty of determining at mitotic metaphase whether the width of 1 or 2 chromatids was being observed. In general, species with shorter chromosomes also had more slender chromosomes, as is apparent in the drawings. The satellites and the short arms of the acrocentrics often appeared considerably narrower than the longer arms.

In *Schistosomatium douthitti* (fig. 8), the first species studied, the chromosome complement consisted of 4 pairs of large to medium metacentric chromosomes which were designated 1 to 4 in order of diminishing length; 2 medium-sized acrocentrics, 5 and 6, the shorter of which (6) bore a satellite, associated with the nucleolus, on the short arm; and a small, often dot-shaped chromosome, 7. In this system, the X is chromosome 1. Although the same relative size relations do not obtain in all other species, numbers were assigned to give the best correspondence to the karyotype of *S. douthitti.* Karyotypes of the other species are briefly characterized below.

Schistosoma mansoni (figs. 1–4): $2n = 16$. Male and female complements alike at mitosis. Chromosomes as follows; No. 1, metacentric; No. 2, acrocentric, secondary constriction about the middle of the long arm; Nos. 3 and 4, metacentric; Nos. 5 and 6, acrocentric; No. 6 with a relatively large satellite on the short arm, and a little shorter than No. 5; No. 7, acrocentric; No. 8, metacentric.

At first, the positions of chromosomes 1 and 2 in the idiogram were uncertain, but a study of pachytene chromosomes (to be published elsewhere) suggests that the metacentric chromosomes correspond to the sex chromosomes of *S. douthitti* and *O. canaliculata;* they are henceforth designated chromosome 1.

All snails infected with *S. mansoni* were laboratory-reared and had been exposed to single miracidia; the sex of each infection had been determined by mouse exposure. Material was studied from 3 geographical sources. There appeared to be little or no difference between karyotypes of the 3 strains. The chromosomes of the Puerto Rican stock were somewhat larger than those in the Egyptian and Liberian stocks, but since the inter- and intrachromosomal proportions were similar in all 3 karyotypes, it appears probable that the size differences are due to environmental influences, possibly from the hosts.

Schistosoma haematobium (fig. 5): $2n = 16$. Male and female complements similar. Karyotype virtually identical with that of *S. mansoni,* except that the satellite on chromosome 6 is smaller.

Chromosomes were studied in cercarial embryos from laboratory-reared snails exposed to single miracidia. The infections were sexed in hamsters.

Schistosoma japonicum (figs. 6, 7) : $2n = 16$. Only 1 karyotype found. Karyotype very similar to those of *S. mansoni* and *S. haematobium,* except that the short arm of chromosome 2 is slightly longer in *S. japonicum,* and the relative lengths of chromosomes 5 and 6 are reversed, i.e., the satellite is on the longer rather than the shorter of the 2 medium-sized acrocentrics, suggesting that a translocation may have occurred between 5 and 6.

Natural infections of unknown sex were used for this study. Attempts to obtain additional material, including sexed snail infections, have been unsuccessful despite several efforts generously made by the Philippine workers and Dr. R. E. Kuntz.

Ornithobilharzia canaliculata (figs. 9, 12, 17–20) : $2n = 16$. Females heterogametic. Chromosomes of the males as follows: No. 1 (X), metacentric, with a secondary constriction in the longer arm in about 20 percent of the chromosomes observed, the region proximal to the constriction frequently but variably faintly stained; No. 2, submetacentric, the short arm about one-half the length of the longer one, a secondary constriction present about the middle of the long arm in approximately 50 percent of the chromosomes observed; Nos. 3 and 4, metacentric; No. 5, acrocentric; No. 6, slightly shorter than 5, acrocentric and bearing a small satellite on the short arm; No. 7, acrocentric; No. 8, metacentric. Chromosomes of the female identical, except that one of the X chromosomes is replaced by a medium-sized rod (Y) having a very minute short arm, less than half the length of the short arm of chromosome 5, with which it might otherwise be confused.

Snails used were all naturally infected. That the 2 different karyotypes corresponded to females (XY) and males (XX), respectively, was suspected by analogy with the situation in *S. douthitti,* and was confirmed by exposing 5 pigeons and 1 parakeet to cercariae from 6 infected snails, 3 with each of the 2 karyotypes. Cercariae with homomorphic karyotypes produced only adult male worms (1, 6, and 59 worms per bird), and those with heteromorphic karyotypes only female worms (1, 2, and 13 per bird) (table I). Additional and perhaps more conclusive proof was furnished by examination of mitotic chromosomes in adult worms (table III; figs. 17, 18).

TABLE III. *Mitotic chromosomes in adult* Ornithobilharzia canaliculata *from pigeons exposed to naturally infected* Batillaria minima.

Sex	Source	No. plates	Mitotic chromosomes ($2n$)
Male	Spermatogonia of 13 worms	31	16, XXAA
Female	Vitellaria of 15 worms	91	16, XYAA
	Ovaries of 12 worms	32	16, XYAA

The X and Y chromosomes are similar to those of *S. douthitti,* except that the X of *O. canaliculata* is relatively shorter and the Y chromosome has a visible, though very tiny, short arm. Careful observation of the Y chromosome of *S. douthitti* in many cells, both at mitosis and at pachytene, has so far failed to yield convincing evidence of a second arm, indicating that the centromere of the Y must be terminal or very nearly so in this species.

Trichobilharzia physellae (fig. 14) : $2n = 16$. Only one karyotype. A single plate showed 17 chromosomes, apparently trisomic for 1 of the smallest elements. Chromosomes as follows: Nos. 1, 2, 3, and 4 metacentric, with No. 1 having a secondary constriction about the middle of one arm; Nos. 5 and 6 subacrocentric,

No. 6 bearing a satellite on the short arm; Nos. 7 and 8 small, probably both with median or submedian constrictions.

The snails studied contained natural infections of unknown sex. Repeated efforts to sex infections by bird exposure were unsuccessful. However, the absence of a heteromorphic pair of chromosomes in material from 11 snails suggests that this species does not possess sex chromosomes distinguishable at mitotic metaphase.

Trichobilharzia stagnicolae (figs. 15, 16). Fifty-eight plates were analyzed in 4 natural snail infections of unknown sex. In 2 snails, 6 and 9 analyzable plates, respectively, contained 18 chromosomes. In the 3d snail, 16 chromosomes were counted in each of 14 plates, while in the 4th snail 16 plates had 18 chromosomes, 13 cells contained 16 chromosomes, and 3 appeared to have 17 chromosomes. The karyotype with 16 chromosomes was designated *T. stagnicolae* A and that with 18 chromosomes, *T. stagnicolae* B. The 3 plates in which 17 were counted may actually have contained 18; they differed from those with 18 by showing only 5 instead of 6 small chromosomes, the ones most easily obscured.

The karyotype of the A material is similar to that of *T. physellae,* differing chiefly in that chromosomes 2, 5, 6, and 7 are somewhat smaller. The B karyotype differs from the A in having an additional small pair of chromosomes (No. 9), and perhaps a slight further reduction in size of chromosomes 5, 6, and 7.

No evidence of a heteromorphic pair of chromosomes was detected in any of the 58 plates examined.

Austrobilharzia variglandis (fig. 11): $2n = 16$. Only 1 karyotype found. Chromosomes as follows: Nos. 1, 2, 3, and 4 metacentric, No. 1 with a secondary constriction near the end of one arm in occasional cells, No. 4 bearing a satellite associated with the nucleolus at mitotic prophase; Nos. 5 and 6 acrocentric, neither having a satellite; No. 7 acrocentric; No. 8 metacentric; 7 and 8 both relatively larger than in most other species.

A. variglandis differs from all other species studied in having the satellite on chromosome 4 rather than on 6. The karyotype is otherwise rather similar to those of *T. physellae* and *T. stagnicolae* A.

A single naturally infected snail furnished material for study of chromosomes of this species, and one parakeet exposed to very few cercariae yielded 2 male worms. The evidence is thus insufficient to determine whether this species has differentiated sex chromosomes.

Gigantobilharzia huronensis (fig. 10, 13): $2n = 16$. In the single naturally infected snail available for study, 24 plates were analyzed, and 2 apparently different karyotypes were observed. One, represented by 15 plates, showed no evidence of a heteromorphic pair (fig. 13); except for the smaller size of the chromosomes the karyotype was similar to those of *T. physellae* and *T. stagnicolae* A, with the usual series of 4 pairs of V-shaped chromosomes (1, 2, 3, 4), 2 pairs of acrocentrics (5 and 6) with the shorter (6) bearing a small satellite on the short arm, and 2 pairs of small chromosomes (7 and 8). In 9 other plates only 7 metacentrics were identified. In the 3 clearest such plates, one of the longest V's appeared to have been replaced by a rod-shaped element (fig. 10). Three chicks were exposed to cercariae from this snail, and only female worms (5, 6, and 15 per bird) were recovered.

The evidence suggests that this species may have heteromorphic sex chromosomes similar to those of *S. douthitti* and *O. canaliculata,* and that the single snail

available may have been infected with miracidia of both sexes; absence of male worms in the exposed chicks could have been due to an immature male infection in the snail. However, the question of whether sex chromosomes are present in *G. huronensis* must await further study. Repeated efforts were made in vain to obtain additional infected snails.

DISCUSSION

Our results agree with those of Ikeda and Makino (1936) on *S. japonicum* and Niyamasena (1940) on *S. mansoni* in respect to chromosome number and the failure to detect heterochromosomes. Except for these points, however, comparison of our findings with those of previous authors is of little value, since in most instances only gross size relations of chromosomes were discernible in their work. Niyamasena (1940) noted that the chromosomes of *S. mansoni* consisted of 4 larger, curved elements with the remainder rod-shaped or rounded. The more critical observations of Ikeda and Makino (1936) on *S. japonicum* permitted matching of 8 homologous pairs in both sexes. The complement reported was: 2 large V-shaped pairs, 1 with a median, the other a submedian centromere; and 6 pairs of rod-shaped elements of gradually diminishing length. Our analysis agrees except for 3 of the smaller pairs which actually have median or submedian centromeres instead of terminal or subterminal ones. The fact that the material studied by Ikeda and Makino was from Japan (apparently) and ours from the Philippines might suggest geographical strain differences. This seems unlikely, however, in view of the similarity of idiograms of the 3 strains of *S. mansoni*. It seems more probable that the centromere regions were less distinct in their sectioned material than in our squashes.

To our knowledge the present work constitutes the first on chromosomes in *Ornithobilharzia, Trichobilharzia, Gigantobilharzia,* and *Austrobilharzia.*

Our previous work has demonstrated a heteromorphic pair of chromosomes in the female of *Schistosomatium douthitti,* and the present study establishes that a similar condition exists in *Ornithobilharzia canaliculata.* These schistosomes are, to our knowledge, the only animals in which both X and Y chromosomes have been unequivocally identified cytologically in the female. Female heterogamety has of course been established genetically in lepidopteran and trichopteran insects, some fishes, the axolotl, and birds (White, 1954); the Y chromosome has been described in *Solenobia triquetrella* (Seiler, 1959) and the X in certain birds (van Brink, 1959). The cytological evidence of female heterogamety in 2 genera of schistosomes suggests that this condition prevails throughout the family, but that morphological differences detectable at mitosis between X and Y have not developed in some genera (e.g., *Schistosoma* and probably *Trichobilharzia*).

Since knowledge of schistosome chromosomes is still fragmentary, extensive application of karyotype data to taxonomic problems must await studies of additional species in various genera. Yet the available data indicate certain points of interest. The karyotype of each of the 6 genera represented in our study is distinctive, while in the 2 genera, *Schistosoma* and *Trichobilharzia,* for which data are available on more than a single species, karyotypes are so similar that separation of species by chromosomes would be difficult or impossible.

The occurrence of 2 chromosome numbers (16 and 18) in what was considered to be *Trichobilharzia stagnicolae* is somewhat puzzling and suggests that 2 separate,

but closely related, species may have been involved instead of 2 strains of the same species. The chief difference between the karyotypes of *T. stagnicolae* A and B is the extra pair of smallest chromosomes in B; and, if a single species is represented, the evidence suggests that the small chromosomes may be genetically largely inert. Inertness of small chromosomes is also suggested by the occurrence of female cercarial embryos of *S. douthitti* which were trisomic for the smallest chromosome (7) and yet were apparently developing normally (Short, 1957).

The fundamental similarity of the karyotypes in the 9 species of the 6 genera studied supports the hypothesis that the schistosomes are a monophyletic group. Furthermore, the available data suggest that the ancestral karyotype consisted of 16 chromosomes: 4 pairs of large to medium metacentrics of different sizes; 2 medium-sized acrocentric pairs, one with a satellite; and 2 small pairs.

The principal morphological changes in karyotype accompanying separation of genera seem to have been translocations, inversions, deletions, and changes in the number of smallest chromosomes, either via aneuploidy or by translocation of their arms to longer chromosomes. Such changes, in contrast to polyploidy, have been indicated in the hermaphroditic flukes by the work of Britt (1947), who considered more than 30 species representing 25 genera and 8 families.

With the above chromosomal constitution assumed as ancestral, one may speculate on the visible chromosomal changes which have accompanied differentiation of the genera studied. Species of *Trichobilharzia* and probably *Gigantobilharzia* have remained similar in karyotype to the ancestral condition. In the evolution of *Schistosoma*, the chief change seems to have been a pericentric inversion in chromosome 2. *Ornithobilharzia canaliculata* and *Schistosomatium douthitti* have developed markedly heteromorphic sex chromosomes and the latter has also lost one of the smallest elements. In *Austrobilharzia* a translocation involving the satellite has probably occurred between chromosomes 6 and 4.

In considering relationships of schistosome genera, it is of interest that the avian *Ornithobilharzia canaliculata* is more similar in karyotype to species of *Schistosoma* than is *Schistosomatium douthitti*, a mammalian species. In this connection, it is noted that Dutt and Srivastava (1955) have suggested revision of the genus *Ornithobilharzia*. Besides other changes, they erected the new genus *Orientobilharzia* to include 3 mammalian species (*O. bomfordi, O. turkestanicum* and *O. dattai*) formerly in the genus *Ornithobilharzia*. Le Roux (1958) likewise has suggested that the mammalian parasites *Ornithobilharzia bomfordi* and *O. nairi* be removed from the genus. He placed the former in a new genus *Eurobilharzia* and the latter in *Bivitellobilharzia* Vogel and Minning, 1940. A study of chromosomes of the species involved in this instance and in others might prove helpful in establishing generic boundaries.

Another proposal of Le Roux (1958) involved separation of *Schistosoma mansoni, S. japonicum*, and *S. haematobium* into separate genera. Although the present chromosome studies do not preclude such a revision, they do not support it.

The similarity of karyotypes in the genus *Schistosoma* indicates that chromosomal changes during evolution of the 3 species studied have been largely submicroscopic ones. Detectable chromosomal changes in *S. mansoni* apparently have not accompanied evolution of physiological differences in strains from Africa and Puerto Rico (Files, 1951).

Such information makes it appear unlikely that analysis of mitotic chromosomes will aid in delineating species, subspecies, varities, etc. in the lower mammalian-human schistosome complexes which occur in Africa (Kuntz, 1955); nor does study of mitotic chromosomes seem to offer hope for confirmation of morphological and physiological differences reported among strains of *S. japonicum* from China, Japan, Formosa and the Philippines (DeWitt, 1954; Hsü and Hsü, 1957, 1958a and b). It should be pointed out, however, that study of meiotic chromosomes may aid considerably in solving taxonomic problems involving closely related species, subspecies, and geographical strains. In this connection it would have been of considerable interest to have information on the bivalents of the F_1 generation which resulted from Le Roux's (1954) hybridization of *S. mansoni* and *S. rodhaini.*

It does not seem overly optimistic to predict that chromosomal data on additional species of schistosomes, combined with morphological and life history information, will be of considerable help with problems of phylogeny within this family. Similar cytological investigations among the blood flukes of turtles (Spirorchiidae), from which the schistosomes apparently have been derived (Stunkard, 1923; Byrd, 1939; and others), may furnish information on the type of hermaphroditic fluke which was ancestral to the schistosomes. To date chromosomal studies have been reported on a single species of spirorchid, *Spirorchis magnitestis* Byrd (Jones and Mayer, 1953).

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We are grateful for aid in collecting and identifying *T. physellae* and *T. stagnicolae* given by Dr. W. W. Cort and for the competent technical assistance of Mr. R. B. Holliman during most of this work.

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EXPLANATION OF PLATES

PLATES I AND II

Metaphase chromosomes from cercarial embryos drawn with the aid of a camera lucida from acetocarmine squashes.

PLATE III

Metaphase chromosomes drawn with the aid of a camera lucida from hematoxylin (Gomori) squashes.

PLATE IV

Idiograms.

PLATE I

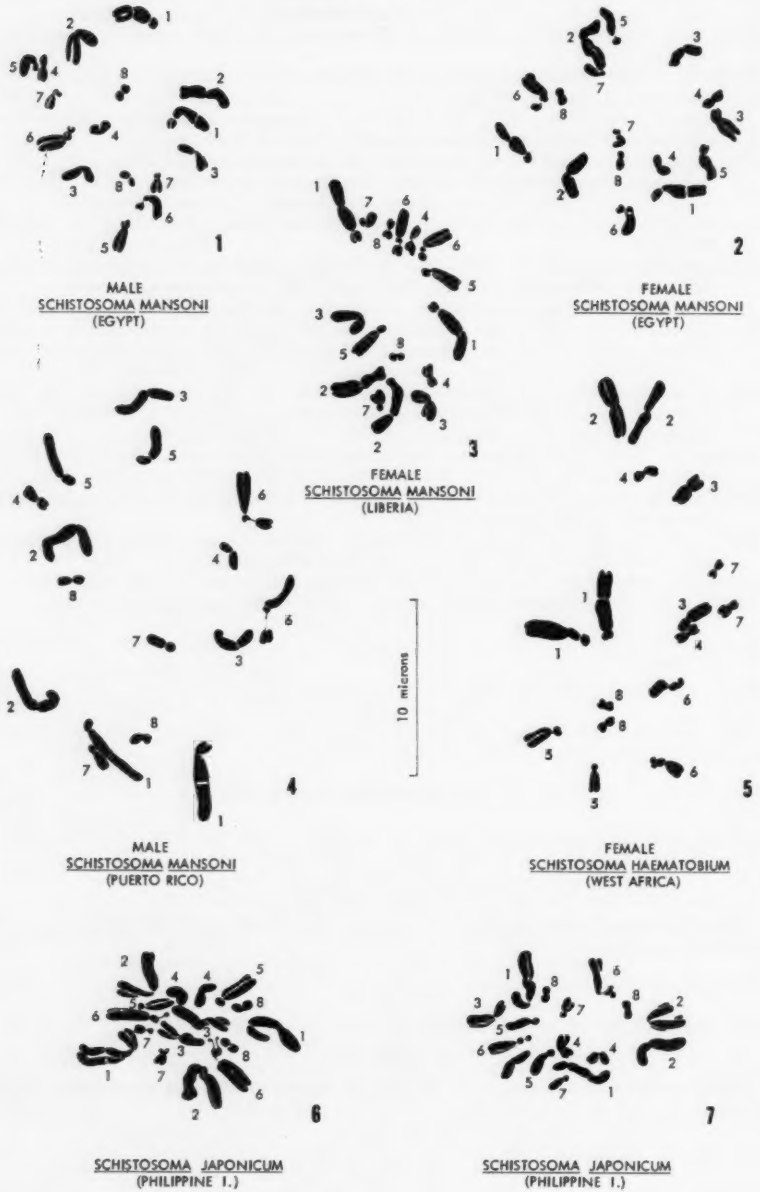
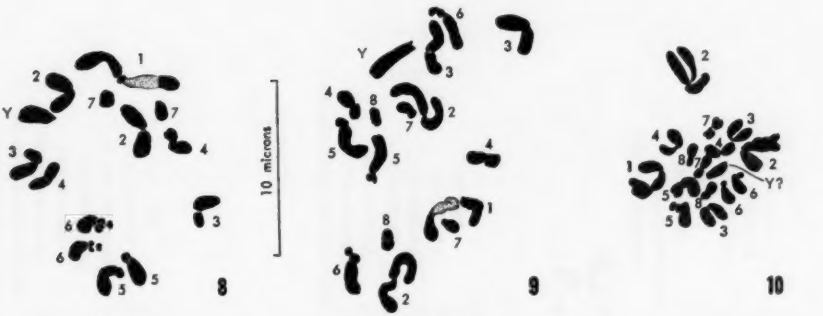

1
MALE
SCHISTOSOMA MANSONI
(EGYPT)
2
FEMALE
SCHISTOSOMA MANSONI
(EGYPT)
3
FEMALE
SCHISTOSOMA MANSONI
(LIBERIA)
10 microns
4
MALE
SCHISTOSOMA MANSONI
(PUERTO RICO)
5
FEMALE
SCHISTOSOMA HAEMATOBIUM
(WEST AFRICA)
6
SCHISTOSOMA JAPONICUM
(PHILIPPINE I.)
7
SCHISTOSOMA JAPONICUM
(PHILIPPINE I.)

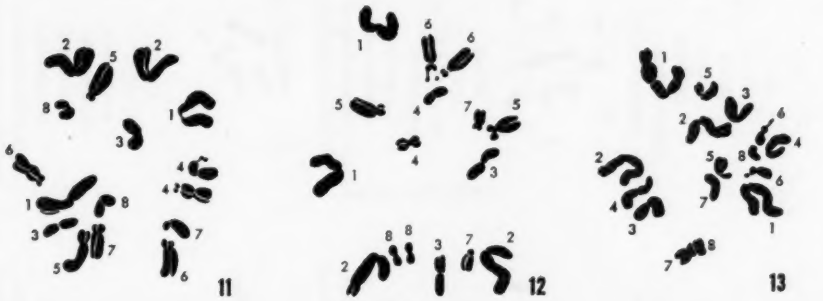
PLATE II



FEMALE
SCHISTOSOMATIUM DOUTHITTI
(MICHIGAN)

FEMALE
ORNITHOBILHARZIA CANALICULATA
(FLORIDA)

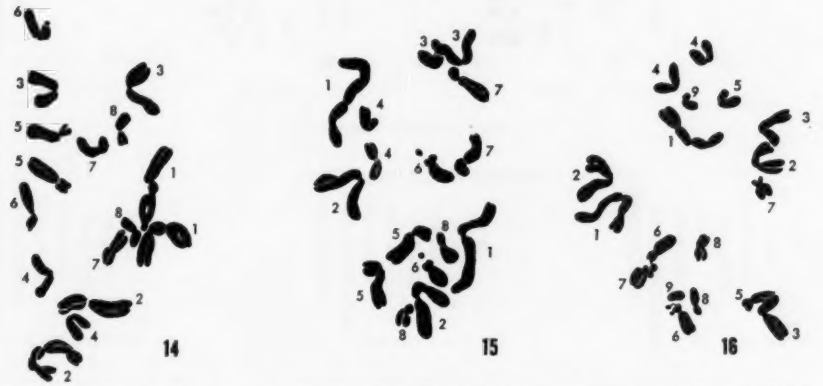
GIGANTOBILHARZIA HURONENSIS
(MICHIGAN)



AUSTROBILHARZIA VARIGLANDIS
(MAINE)

MALE
ORNITHOBILHARZIA CANALICULATA
(FLORIDA)

GIGANTOBILHARZIA HURONENSIS
(MICHIGAN)

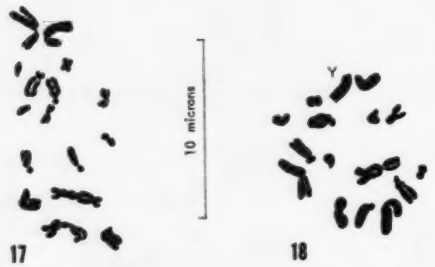


TRICHOBILHARZIA PHYSELLAE
(MICHIGAN)

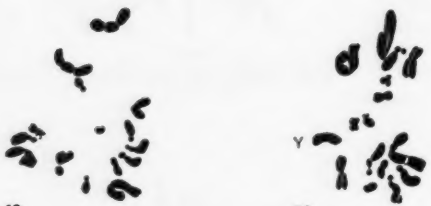
TRICHOBILHARZIA STAGNICOLAE A
(MICHIGAN)

TRICHOBILHARZIA STAGNICOLAE B
(MICHIGAN)

PLATE III



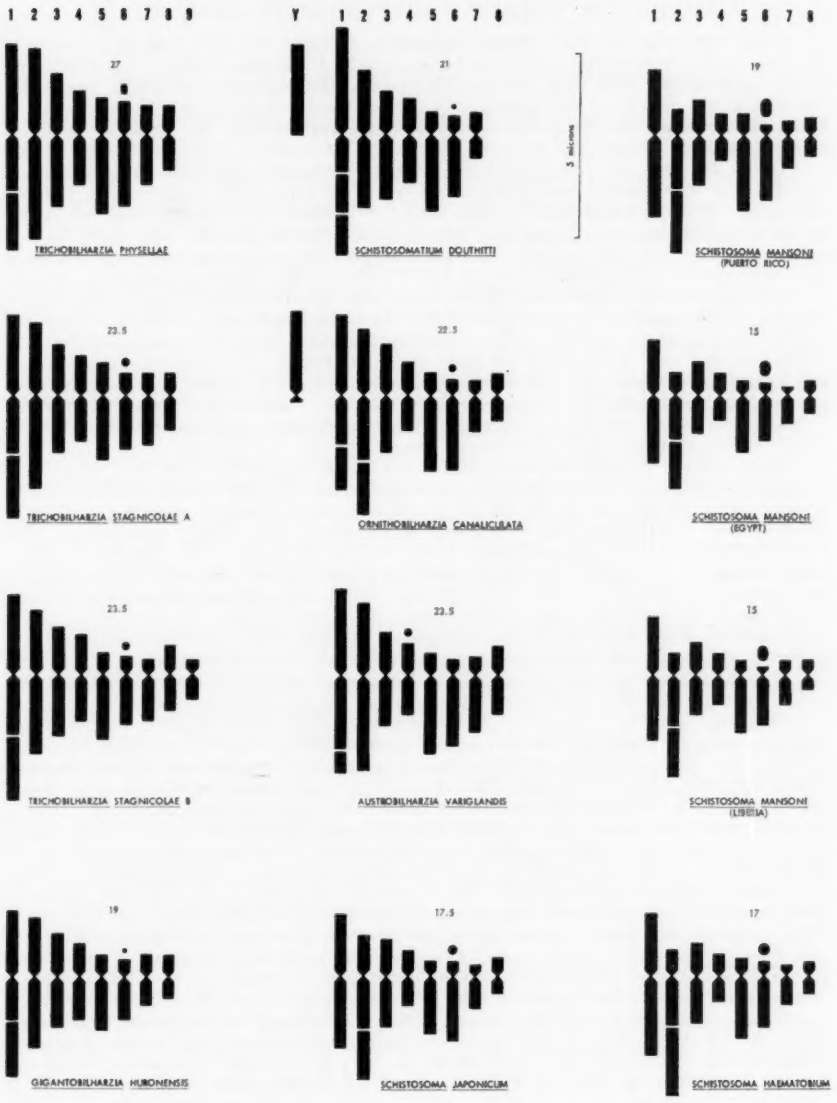
FROM ADULT WORMS



FROM CERCARIAL EMBRYOS

ORNITHOBILHARZIA CANALICULATA

PLATE IV


1 2 3 4 5 6 7 8 9
27
TRICHOBILHARZIA PHYSELLAE
V 1 2 3 4 5 6 7 8
21
SCHISTOSOMATIUM DOUTHITTI
5 microns
1 2 3 4 5 6 7 8
19
SCHISTOSOMA MANSONI
(PUERTO RICO)
23.5
TRICHOBILHARZIA STAGNICOLAE A
22.5
ORNITHOBILHARZIA CANALICULATA
15
SCHISTOSOMA MANSONI
(EGYPT)
23.5
TRICHOBILHARZIA STAGNICOLAE B
23.5
AUSTROBILHARZIA VARIGLANDIS
15
SCHISTOSOMA MANSONI
(LIBERIA)
19
GIGANTOBILHARZIA HURONENSIS
17.5
SCHISTOSOMA JAPONICUM
17
SCHISTOSOMA HAEMATOBIUM

RESEARCH NOTE

A METHOD OF ISOLATING TRYPANOSOMES FROM BLOOD.

It has been found that Bacto-Phytohemagglutinin (Difco, Code 0528) is useful as an aid in removing mature erythrocytes from blood (Li et al, 1949, J. Hematol. **4**: 670–675). The manufacturer recommends using 1 mg, contained in 0.1 ml of the rehydrated solution, to agglutinate the erythrocytes from 10 ml of heparinized or citrated blood. However, it may be desirable to titrate more precisely the optimum concentration of the agglutinin for the particular species of mammal involved.

To test this substance as an aid to the isolation of trypanosomes, blood was drawn by cardiac puncture with a heparinized syringe and needle from C3H mice which had numerous trypanosome stages of *Trypansoma cruzi* in their blood. Rehydrated phytohemagglutinin was added to the heparinized blood to make a final concentration of 0.1 mg per ml. The suspension was shaken gently for 5 seconds and, after allowing it to stand at room temperature for 5 minutes, it was centrifuged at approximately 1600 rcf for 10 minutes. The supernate, containing most of the trypanosomes, was removed and the flagellates were then concentrated by centrifugation at 2800 rcf for 10 minutes. They were then washed by suspension in sterile Locke's solution and sedimented by centrifugation, this process being repeated twice to insure adequate washing. All of the foregoing steps were carried out under aseptic conditions. The trypanosomes isolated in this manner were highly concentrated, appeared to be normal with respect to morphology, and proved to be highly infective for mice and tissue cultures. Counts of the trypanosomes in the original blood sample and in the suspension after isolation and washing showed that 50 to 60 percent of the organisms were recovered in a routine run.

Blood forms of *T. gambiense* were isolated in a similar manner from rat blood diluted 1 : 3 with Alsever's solution (glucose, 4.66 gm; sodium chloride, 1.05 gm; sodium citrate, 2.0 gm; distilled water, 200 ml; pH to 6.5 with 10 percent citric acid). Counts revealed that 70 to 80 percent of the trypanosomes were recovered from the blood. The isolated trypanosomes were frozen by the method of Polge and Soltys (1957, Tr. Roy. Soc. Trop. Med. Hyg. **51**: 519–526). After storage for 8 months at −65 C, the flagellate suspensions were thawed by immersing the glass ampules in tap water at 20 C. Approximately 20 percent of the parasites were motile, and no decrease in virulence was noted when they were inoculated into rats.

This method has been successfully employed in searching blood for trypanosomes in light infections. The organisms in relatively large samples of blood can be concentrated in a drop or two of fluid, thus enabling a rapid diagnosis by direct examination. The concentrated material can be cultured on appropriate media or inoculated into suitable laboratory animals.

Phytohemagglutinin may be useful in isolating extracellular blood parasites for psysiologic studies or antigen preparation. With care the above procedure removes essentially all of the erythrocytes. Many leucocytes and platelets are removed by entrapment in clumps of erythrocytes or centrifugation, but a significant number of non-agglutinated blood cells may remain with the parasites. Antiserum to the cells not agglutinated by phytohemagglutinin was prepared by serial intravenous inoculations of a rabbit with suspensions of these cells obtained from normal rat blood treated with phytohemagglutinin. The antiserum was inactivated at 56 C for 30 minutes before use and was added to the trypanosome suspensions after the erythrocytes had been removed with phytohemagglutinin; the amount of antiserum to be added had previously been determined by titration with suspensions of non-agglutinable cells from normal rat blood. The mixture was incubated at room temperature for 10 to 20 minutes and then centrifuged at 1900 rcf for 10 minutes. In this manner it was possible to remove 99 percent or more of all erythrocytes, leucocytes, and platelets, leaving almost pure suspensions of motile *T. gambiense* which showed no reduction in virulence for rats and appeared to be normal with respect to morphology. Centrifugation at lower temperatures in a refrigerated centrifuge may be necessary where packing and warming at high speeds are detrimental to a particular species of parasite.

(Supported by a research grant (E-977) from the National Institutes of Health.)—ROBERT G. YAEGER, *Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, La.*

THE LIFE HISTORY AND AFFINITIES OF THE TURTLE LUNG FLUKE, *HERONIMUS CHELYDRAE* MACCALLUM, 1902*

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Knowledge of embryological development and larval stages has been generally applied to the problem of relationships among animals and has given strong evidence of affinities between certain groups. In the digenetic trematodes, that knowledge has revealed hitherto unsuspected affinities and has prompted a re-examination of earlier concepts and taxonomic schemes. As a result, several investigators have proposed, for such restricted groups as families and superfamilies, revised concepts which have been incorporated in the comprehensive taxonomic scheme proposed by La Rue (1957). Because information from life history studies is still lacking for many groups, chiefly those occurring in marine hosts, La Rue's scheme is admittedly tentative in respect to such groups. Among trematodes parasitizing freshwater and terrestrial hosts, however, such knowledge is more extensive, so that at present there are few such trematodes whose affinities remain obscure. One is the turtle lung fluke, *Heronimus chelydrae,* whose anatomy is so unusual that the species has long been placed in a distinct family, the Heronimidae, with no apparently close relationships to any other group of trematodes. The present study was prompted by the isolated position of that family and by the occurrence of *H. chelydrae* in several species of turtles in the vicinity of Lafayette, Indiana, thus assuring abundance of material.

Heronimus chelydrae was originally described from the lungs of *Chelydra serpentina* by W. G. MacCallum (1902), who assigned the trematode to Monticelli's family Monostomidae, but recognized its unique differences from other species in that family. Barker and Parsons (1914; 1917) proposed the name *Aorchis extensus* for a lung fluke from *Chrysemys marginata,* and believed by them to be distinct from *H. chelydrae.* To include the genera *Heronimus* and *Aorchis,* Ward (1917) erected the family Heronimidae; he later (1918) emended its diagnosis slightly and stated that because *H. chelydrae* and *A. extensus* were imperfectly known, they might prove to belong to the same genus, if not indeed to the same species. That possibility was confirmed by Stunkard (1919) who compared lung flukes from six species of turtles and reduced *A. extensus* to synonymy with *H. chelydrae.*

G. A. MacCallum (1921) tentatively described two new species, *H. geomydae* from *Geomyda punctularia,* a Trinidad turtle, and *H. maternum* from *Emys blandingi* collected in Ohio. Caballero (1940), however, compared flukes from 16 *Kinesternon hirtipes* with the type specimens of *H. geomydae* and *H. maternum* and concluded that all were identical with *H. chelydrae.* That species would, thus, be the sole representative of the family Heronimidae.

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Lynch (1933) published a detailed study of the miracidium of *H. chelydrae* obtained from snapping turtles in the vicinity of Stillwater, Oklahoma. Sizemore (1936) found that large turtles from that area harbored fewer but larger worms than did smaller hosts. More recently, Guilford (1955) studied gametogenesis in adult flukes obtained from turtles in Wisconsin.

During the summer of 1956, the writer began an investigation of the life cycle of *H. chelydrae* and found that the miracidium penetrated *Physa integra* and developed into a sporocyst which produced cercariae without an intervening sporocyst or redial generation. Late that summer, he learned that two other workers were independently investigating the life cycle of *H. chelydrae*. From their work, two papers have appeared, one by Guilford (1958) on the germinal cell cycle in the miracidium and sporocyst and the other by Ulmer and Sommer (1957) who described the development of the sporocyst in the intermediate host. As their observations on the remainder of the life history had not progressed as far as those of the writer's, they generously agreed that the completion of the study would be left to him.

MATERIALS AND METHODS

Adult trematodes were obtained from naturally infected turtles. These were decapitated, and their lungs were opened and washed in 0.7 percent NaCl solution. At first, miracidia were obtained from eggs which hatched in water soon after their release from the terminal portion of the uterus by dissection of the worm. Later, it was found that if fully mature worms were placed in water, the eggs would hatch in utero and the miracidia emerge from the genital pore.

Various mollusks, collected from a pond where naturally infected turtles occurred, were exposed to miracidia and watched under the dissecting microscope for evidence of penetration. After the molluscan host species was determined, it was reared in the laboratory in battery jars filled with aerated pond water. Lettuce was found to be better food under laboratory conditions than materials from the habitat of the snail. Developmental stages of the parasite were obtained by infecting both laboratory-reared and field-collected snails. Varying numbers of miracidia were used, but 5 to 10 per snail were necessary to insure a high incidence of infection. Developing sporocysts were obtained by removing the shell of the snail and dissecting the mantle region in 0.2 percent saline. Cercariae also were obtained in that manner after it was found that they did not emerge spontaneously from the molluscan host.

Stages of the life cycle were studied as both living and prepared material. To observe development of the cercarial excretory system, sealed perparations of living embryos in weak saline solution or egg white were studied under the oil immersion lens. Neutral red was used as an intravitam stain for cercariae and miracidia. Epithelial plates of the miracidium were demonstrated by the method of Lynch (1933).

Whole mounts of various stages were fixed in corrosive-acetic or alcohol-formalin-acetic fixatives under light cover glass pressure and stained with either Semicon's carmine or hematein. Sectioned material was stained with hematoxylin and eosin. Drawings were made by microprojection, certain details added free hand, and all measurements expressed in millimeters.

RESULTS

Experimental Demonstration of the Life Cycle

A local pond from which infected turtles had been collected for many years was selected for study of the ecology and life history of *H. chelydrae*. Mollusks also had been collected and examined for larval trematodes during that period. Without exception, cercariae that emerged spontaneously either were species whose life histories were known or were not of the type that could conceivably be the larva of *H. chelydrae*. For that reason, it was decided that the most effective approach would be to find a host that the miracidium would penetrate. Toward that end, all species of mollusks that could be found in the pond were collected and exposed to infection. It was learned that the miracidia of *H. chelydrae* attempted to penetrate

only the snails *Helisoma trivolvis* and *Physa integra,* and were successful in penetrating and developing in only the latter.

After *Physa integra* was determined to be the probable intermediate host, several groups of that species were collected in the field, exposed to miracidia in late June and early July, and dissected at intervals to observe the development of the sporocyst. The incidence of infection among these groups was from 50 to 100 percent. It was discovered that after penetration the miracidium shed the epidermal cells and developed into a miracidium-sporocyst which retained the eyespots. Initially, that stage was simple, but within 10 days it showed pulsatile outpocketings which later became permanently formed pairs of lateral processes.

The embryos that are already present in the miracidium grow very rapidly in the young sporocyst, so that well-formed amphistomate cercariae may be present in 17 days. Development to that point is delayed about a week in snails infected with several sporocysts. There is no evidence of a larval generation between the miracidium-sporocyst and cercaria as in most of the digenetic trematodes.

Infected snails were maintained in the laboratory for over 70 days, but cercariae were never observed to emerge from any but moribund ones. However, cercariae mechanically released from the snail swam actively in pond water or crawled for several hours. On release from the mantle tissue, many lost their tails and moved rapidly over the substrate in an inchworm manner.

The infection in the intermediate host matures rapidly and is of short duration, compared with most digenetic trematodes. Snails dissected 6 weeks after infection often contained either no trace of the parasite or only a few cercariae and remnants of the sporocyst. Few snails showed active cercariae longer than 2 months after exposure to miracidia, and then only in small numbers. The short duration of the infection in the snail combined with failure of the cercariae to emerge led to the conclusion that the next host must obtain the parasite by eating the infected snail during the rather limited period that it contains cercariae.

What seemed to be abundant cystogenous glands in the cercaria suggested that the life cycle of *H. chelydrae* included an encysted metacercaria, either on vegetation or in a specific second intermediate host eaten by turtles. However, the cercariae exhibited no tendency to encyst on vegetation or the dish when liberated mechanically, nor did extensive examination of animals that might serve as a second intermediate host reveal any infections that seemed at all likely to be metacercariae of *H. chelydrae.* Furthermore, encystment did not occur in any of a variety of animals collected from the pond and exposed to cercariae liberated from the snail.

Failure to demonstrate an encysted metacercarial stage seemingly left but the one alternative, the direct infection of turtles by eating snails. To test that possibility, a large colony of laboratory-reared snails was used. In late June and early July, 1958, they were exposed to miracidia; practically every snail became infected. They were fed to turtles after verifying the presence of mature sporocysts which could be seen through the intact shell. As laboratory-reared turtles were not available, small ones that had been held in captivity for at least 2 months were used.

Each of 4 *C. serpentina* was placed in pond water with several snails harboring 30- to 40-day experimental infections. All 4 ate the snails, and were later sacrificed and examined. Two became infected; one, examined 1 day after exposure, had 31 trematodes in the posterior region of the lungs and the other, examined after 3 days, had 10. In both cases the parasites differed from the cercariae only in lacking

the tail and in having the intestinal cecal distended with ingested blood. Similar results were obtained with 5 *C. picta marginata*, 2 of which became infected. None of the turtles harbored specimens that could be attributed to previous natural infections.

In the next experiment, infected snails were fed to 3 *C. picta marginata* to determine the route whereby the parasite reaches the lungs. In a turtle examined 30 minutes later, the stomach contained the intact snail in which active cercariae were present. In another examined 2 hours after feeding, the snail was found in the stomach with the shell broken and the cercariae being released; 2 active cercarial bodies were found in the small intestine and 2 in the trachea. The third turtle was sacrificed 20 hours after feeding. Shell fragments and the partially digested snail were in the intestine, but a complete examination revealed no cercarial bodies.

In another experiment, cercariae were blown down the esophagus of 9 *C. picta marginata* by means of a pipette. In 1 turtle, killed 30 minutes later, 5 cercariae were found in the esophagus and 2, already without tails, in the trachea. These cercariae, like all others found within a few hours after infection, were crawling in an inchworm manner, using the suckers alternately for attachment to the walls of the digestive or respiratory tract. Another turtle, examined 1 hour after cercariae were forced down the esophagus, was negative, but a third examined after 2 hours, had 1 cercarial body in the bronchus and 1 in the lung, but none elsewhere. The remaining 6 turtles were held for a longer time and examined; 3 of them were infected.

The above experiments indicated a tracheal rather than a parenteral route of the parasite to the lung and a considerable risk of failure to reach the lungs if the infected snail is swallowed. To minimize that risk, cercariae were blown directly down the trachea of 7 *C. serpentina*. They were examined at intervals of up to 3 months; all were found to be infected with *H. chelydrae*. Two harbored large worms from previous natural infections, but all contained also young flukes whose age, as estimated by size and development, corresponded to the length of time that elapsed between exposure and examination of the hosts.

The above experiments show that the cercariae reach the lungs by way of the trachea from either the mouth or upper digestive tract. They probably cannot migrate from the lower intestine, since those found in the duodenum were usually dead and partly digested.

An explanation of the small number of worms usually present in naturally infected turtles is provided by the higher incidence and intensity of experimental infections in which cercariae were placed in the trachea as compared with infections obtained by migration of the larvae from the digestive tract. As turtles usually feed, infected snails may be swallowed more or less intact, and, as experimental feeding has demonstrated, few of the cercariae would be expected to find their way from the stomach to the lungs. Should the infected snail be crushed in the mouth, however, the risk of failure to reach the lungs would be much less, thus explaining the occasional heavy natural infection observed. Such infections could result also from ingesting more than one infected snail. Another factor that could affect both the incidence and intensity of infection in turtles is the relatively short period during which cercariae remain in the snail. Toward the end of that time, very few are present and, if the snail containing them is swallowed by a turtle, few if any of the larvae would be expected to reach the lungs.

The incidence of snails naturally infected with *H. chelydrae* is very low in the pond used for this study. Hundreds of *Physa integra* from that source were cracked and examined throughout the summers of 1956 to 1958, but a natural infection with *H. chelydrae* was not found until October, 1958, when 1 of a lot of 150 snails yielded 2 large sporocysts.

One phase of the life history of *H. chelydrae* remained to be determined, viz., the manner of infection of the snail host under natural conditions. As a rule, the eggs of digenetic trematodes are voided in the host's feces. However, repeated examination of scrapings and washings from the lungs, respiratory passages, and digestive tract of infected turtles failed to reveal any miracidia or eggs. Moreover, flukes gravid with many eggs containing fully developed miracidia were never observed to deposit them when removed from the host and placed in 0.6 to 0.7 percent NaCl, but if transferred to pond or distilled water, the miracidia hatched in utero and emerged through the genital pore. These observations indicate that *H. chelydrae* does not deposit eggs in the lungs, nor do the miracidia hatch within the fluke until it reaches a hypotonic environment. Instead, there is evidence that the gravid worm must migrate from the lungs and reach water to release the miracidia. On several occasions, particularly in early summer, such worms were found in the bronchi, trachea, and once just within the glottis of naturally infected turtles that were killed and examined.

Stages in the Life History

The adult. The morphology of the adult has been adequately described by MacCallum (1902) and Stunkard (1919), and will not be repeated here. In this study, the number of parasites per infected turtle varied from 1 to 61, with an average of about 5 in *C. picta marginata* and *C. serpentina.* Of 40 snapping turtles examined, 70 percent were infected, whereas only 35 percent of 253 mature painted turtles were found to harbor *H. chelydrae.* Sizemore (1936) reported an incidence of 68 percent in snapping turtles from Oklahoma and Ulmer and Sommer (1957) 28 percent of 46 *C. picta belli* and 43 percent of 7 snapping turtles in Iowa. Other species of turtles collected near Lafayette were occasionally found to be infected, but too few were examined to provide an accurate estimate of the infection rate. No difference of incidence in male and female turtles was observed.

The largest flukes obtained from naturally infected turtles were about 15 long and 1.5 wide, the smallest less than 1.0 in length. The genital complex apparently becomes functional when the fluke attains a length of about 3.0; the smallest worms observed to contain developing embryos were slightly over that length. An infected turtle usually harbored worms of rather uniform size, possibly from eating a single infected snail, but sometimes 2 or 3 distinct size groups were present.

Seasonal incidence and maturity of natural infections were followed in *C. picta marginata,* the only species trapped in numbers throughout the collecting season from May to October. Gravid worms, i.e., ones containing miracidia that hatch readily, were abundant in June and July, but scarce in August and September; usually they were at least 10.0 long, but occasionally, during June and July, some as short as 6.0 or 7.0 yielded eggs that hatched normal miracidia. In large gravid worms, most of the uterus contained fully embryonated eggs. Worms over 10 in length found during August and September and before June contained no miracidia that would hatch. In August and September, terminal portions of the uterus in such

large worms were usually either empty or filled with dark eggs containing degenerate miracidia. As a rule, worms found prior to June yielded no eggs that would hatch. Miracidia were obtained from a specimen late in May, but they were sluggish and died in a short time. Although immature worms were found throughout the collecting season, worms less than one month old, as judged by comparison with those found in experimental infections, were found only in August and September.

Thus, the data on natural infections at various times of the year indicate that (1) the life cycle of *H. chelydrae* in *C. picta marginata* is decidedly seasonal with the peak of gravid specimens occurring during June and July in North Central Indiana; (2) size alone does not determine the presence of eggs that hatch fully developed miracidia; (3) the incidence of the infection does not vary greatly from month to month; and (4) very young worms are present only in late summer.

No gross pathological changes were obvious in naturally infected turtles. In heavy experimental infections, however, the lungs were often noticeably mottled with dark pigment, probably hemosiderin. Sections of heavily infected lung tissue from a small *C. serpentina* revealed concentrations of pigment around the blood vessels and in the subepithelial tissues. No evidence of its being intracellular was observed. As there was no indication of extensive hemorrhage, it was concluded that the source of the pigment probably is the residue of digested blood regurgitated by the parasite. Worms of all sizes feed heavily on blood, and it is always present in the intestine, even shortly after the cercarial bodies reach the lungs. As the worms increase in size several times before attaining maturity, they probably ingest a great deal of blood. There appeared to be little to no tissue reaction to the presence of either the pigment or the trematodes which were often within the terminal respiratory passages in intimate contact with the lung tissue. There may be a slight hyperplasia of the respiratory epithelium, but heavily infected turtles were always active and showed no evidence of respiratory difficulty.

The egg and miracidium. Fully embryonated eggs removed from the uterus usually hatch within 1 to 5 minutes when placed in pond water. The miracidia become active immediately and swim rapidly in circles within the swollen eggs, probing at the shell with the terebratorium. As the shell is thin and lacks an operculum, it splits on hatching. Eggs placed in 0.5 percent to 0.8 percent NaCl solutions do not hatch, but may remain there at least 3 hours, and then promptly hatch when transferred to pond water or NaCl solutions of lower concentrations. The miracidia are rather inactive within the eggs as long as they remain in 0.5 percent to 0.8 percent NaCl. Their increased activity at lower osmotic pressures appears to cause splitting of the egg shell; whether the penetration glands function in the hatching process could not be determined by observation. Hatching occurs equally well in the light or dark.

Study of both living and fixed miracidia has revealed almost complete agreement with the detailed description given by Lynch (1933). However, the extent of variation in the number of flame cells and the epidermal plate pattern reported by him was not found to be as great in the present study. The author observed only 2 flame cells in both miracidia and the many young sporocysts that were studied. The epidermal cell pattern as delineated by the silver nitrate method was determined in 142 miracidia. Most of the variations reported by Lynch were found, but were not as frequent as he observed. Only 8 miracidia had a pattern of other than 6:9:4:2, and the 6:8:4:2 arrangement reported for paramphistomes

(Bennett, 1936; Willey, 1941) was found only once.

The miracidium of *H. chelydrae* is unusual in the extent to which germinal development is advanced and the relationship of its germinal elements to the soma of the larva. Its center is occupied by a thin sac containing embryos showing a distinct anterior-posterior gradient in their size and number of cells (fig. 1). The embryos are largest anteriorly and, according to Lynch (1933), may attain a maximum size of 0.059 by 0.038; posteriorly, they are smaller and many consist of only 2 or 3 cells. The largest embryos contain a lumen, have a thin investing membrane, and are separated by delicate partitions extending inward from the sac. Anteroventrally, the sac is attached to the body wall by a group of cells; posterodorsally, it bears a tail-like appendage which contains the smallest embryos and turns dorsally to end against the body wall of the larva.

Several miracidia have been described in which germ balls, i.e., embryos, are present. In *Zygocotyle lunata,* Willey (1941) found 1 or more germ balls up to 0.025 in diameter and composed of about 16 cells. Bennett (1936) also reported small germ balls in the miracidium of *Cotylophoron cotylophorum.* Some investigators have found that the germinal elements of miracidia are not free in the body cavity, but are attached by strands to the body wall (Price, 1931). However, the well developed sac enclosing those elements in the miracidia of *H. chelydrae* seems to be unique.

An interpretation of that sac is suggested by such genera as *Stichorchis, Parorchis,* and *Typhlocoelum* in which the miracidium contains a fully formed redia on hatching. As *H. chelydrae* is the only paramphistomoid known to lack a redial generation in the life cycle, the sac may well represent that generation in an incipient or, more likely, retrogressive state. It may be significant to that interpretation that *Stichorchis* is a typical paramphistome and that other genera in which the miracidium contains a redia probably are not distantly related to the paramphistomes. Furthermore, in the miracidium of *H. chelydrae,* the germinal elements are arranged in the sac in a manner suggesting that found in the redia of a number of trematode families, particularly Paramphistomidae and Notocotylidae; however, the embryology of the miracidium, as described by Guilford (1958), provides no support for the interpretation of the sac as an incompletely developed larval generation.

After hatching, the miracidium remains active for 4 to 6 hours in pond water at room temperature and is positively phototactic. It was not attracted to the snail; instead, penetration followed random contact with the host and often required several attempts at various places, usually the tentacle and mantle region. Penetration time varied with the part attacked, the miracidium requiring but 2 to 4 minutes to enter the tentacle and as long as 30 minutes to penetrate the foot where penetration was often not successful. In one snail removed from the shell, the miracidia showed no tendency to enter the delicate surfaces thus exposed, but penetrated instead the parts normally attacked. The host often reacted violently to the penetrating miracidia, apparently in an effort to dislodge them, and sometimes withdrew the body into the shell.

The epidermal plates of the miracidium were not lost immediately upon penetration, but remained intact for a short time. In one case, their shedding was observed in a miracidium removed 20 minutes after entering the snail. By 24 hours, the larvae without epidermal plates were usually found beneath the mantle or just behind the buccal mass.

The miracidium-sporocyst (figs. 2 to 8). Within the snail, the miracidium develops rapidly to become the miracidium-sporocyst which, even when very small, is easily recognized by its activity and the eye-spots which are retained. The miracidium-sporocysts are not attached; the host's tissue needs merely to be torn to release them. Not all of the miracidia that penetrate the snail are able to develop further. The number of sporocysts per infection varied from 1 to 25, but averaged less than 5, even in snails exposed to large numbers of miracidia. In snails from 4 to 9 mm in length of shell, heavy infections developed in only those near the maximum size.

Growth and development of the sporocyst as observed in experimentally infected snails was substantially as Ulmer and Sommer (1957) have described and need not be repeated here, but figures of the miracidium and sporocyst at various stages are included. Certain features apparently not observed by Ulmer and Sommer should be mentioned. Although the 2 large miracidial flame cells and their ducts persist in the sporocyst, it was observed that by as early as the 10th day several smaller cones of cilia appeared near each of the original flame cells (fig. 3). In sporocysts 15 to 20 days of age, there may be as many as 100 of the smaller "flame cells" which remain in the vicinity of the large ones but become more widely dispersed than in younger sporocysts.

As early as the 17th day, well developed amphistomate cercariae were found beneath the mantle of a few snails and within the sporocysts. Ulmer and Sommer (1957) described birth pores at the tips of the sporocyst branches through which the progeny emerged, and in the present study the invaginated tips of many branches had the appearance of pores. However, careful examination of fully expanded branches did not reveal such openings. Instead a single mid-ventral pore just posterior to the level of the eye-spots was observed in 16-day-old sporocysts. In older ones the pore was seen to open and close, but no embryos were observed to emerge except when the sporocyst was handled roughly, causing them to escape there and elsewhere due to rupture of the wall. Nevertheless, it seems likely that the single median pore is the means by which the progeny normally escape from the sporocyst.

The miracidium-sporocyst of *H. chelydrae* does not closely resemble that of any other trematode. Its bilateral symmetry with a nearly constant number of paired branches, retention of miracidial eye spots, and features of the excretory system suggest a larval stage more uniform in shape and of somewhat greater structural complexity than a miracidium-sporocyst in the usual sense. The situation is not the same as in such families as the *Bucephalidae* and *Brachylaemidae* in which that stage branches in an irregular manner as it grows through the host's tissues. Also, in those families, embryos develop from germinal cells in the branches which thus increase the reproductive potential of the sporocyst. In fact, Ciordia (1956) suggested that in the bucephalid *Rhipidocotyle papillosum* the branches represent a second larval generation which remains attached to the parent. In *H. chelydrae,* however, they have no germinal function but merely increase the space that the embryos may occupy.

The cercaria and the embryology of its excretory system (figs. 9 to 19). Cercariae do not normally emerge from the snail, thereby indicating an endpoint of their development in that host. To judge from their size, activity and structure, however, cercariae in snails containing 1 or 2 sporocysts are fully developed and prob-

ably infective by the 22nd day after penetration by the miracidium. Occasionally, cercariae of full size are present as early as the 17th day, but lack cuticular spines and often are not active on removal from the snail. In multiple infections, development is somewhat retarded; but by 30 days most infected snails contain large, active cercariae. No attempt was made to establish by experimental feeding the exact age at which cercariae become infective.

In the following diagnosis of the cercaria, measurements are from 10 larvae fixed in hot 3 percent formalin; 5 were from a 32-day infection, and 5 from a 52-day one.

Amphistomate larvae with well developed suckers and tail; ventral sucker a short distance from body-tail furrow. Body 0.487 to 0.501 long; rather uniform in width between suckers, width at ventral sucker 0.165 to 0.210. Tail broadly attached to posterior end of body, 0.51 to 0.60 long, with basal swelling 0.080 to 0.150 wide; width at mid-length 0.060 to 0.112; posterior end usually somewhat enlarged, with a pointed tip. Cuticle of entire body and basal swelling of tail with small spines in quincunx, larger and denser around sucker openings except dorsal lip of mouth which lacks spines. Eyespots and other pigment absent; body with numerous glands, some within oral sucker with ducts opening at anterior margin of body, others having the appearance and distribution of cystogenous glands; basal enlargement of tail with a cluster of large hyaline cells, with strands attached to periphery of tail. Oral sucker subspherical, 0.111 to 0.142 in diameter, mouth subterminal; ventral sucker 0.099 to 0.118 in diameter. Prepharynx very short; pharynx 0.036 to 0.051 in diameter; esophagus 0.017 to 0.052 long; ceca thin-walled, ending blindly at anterior margin of ventral sucker or slightly overlapping that level. Lateral ganglia and commissure dorsal to esophagus very conspicuous. Genital primordium an elongate, irregular mass 0.032 to 0.048 long, 0.016 wide, median, near dorsal surface a short distance anterior to ventral sucker. Excretory vesicle thin-walled, saccate, mostly between ventral sucker and body-tail furrow; definitive pore dorsal; primary pores at sides of tail very close to its tip, connected to bladder by long caudal tubule which may become degenerate. Anteriorly, excretory vesicle tapers abruptly to a median tube which bifurcates just anterior to ventral sucker to form a pair of voluminous branches extending to pharyngeal level; each branch with 15–22 refractile concretions up to 0.017 in diameter, composed of concentric layers; from anterior end of each branch a recurrent tubule extends posteriorly almost to level of ventral sucker where each receives an anterior and posterior secondary tubule, each joined by capillaries from 2 flame cell groups; flame cells of posterior group crowded, in basal enlargement of tail; flame cell formula $2\,[(7+7)+(7+6)]=54$ flame cells. Host: *Physa* spp.

When mechanically released from the snail, cercariae swim and crawl intermittently for at least 8 hours in 0.2 percent saline or pond water at room temperature. In swimming, the tail is turned ventrally until almost parallel to the body and lashed vigorously. The larvae do not swim smoothly and decaudation frequently occurs. Active cercariae often attach to any submerged object and crawl for long periods before swimming is resumed. When crawling, the cercariae attach firmly to the substrate by the suckers and are not easily dislodged.

The developing excretory system of the cercaria is first seen as a pair of flame cells with separate ducts opening laterally near the posterior end of small ovoid embryos (fig. 12). In slightly larger ones, the flame cell number has increased to 4 and the ducts have turned laterally to receive their capillaries (fig. 13). With further development, the primary ducts become more incurved, come close together and fuse (figs. 14 and 15); the stenostomate condition is obvious with the recurrent tubules turning posteriorly before receiving the capillaries from the flame cells. By the time the tail primordium is evident, there are 3 pairs of flame cells, with the posterior pair within that primordium or close to its base, and the developing suckers and pharynx are distinct (fig. 16). As the tail increases in length, the caudal excretory tubule is carried with it to open laterally at paired pores near the tip of the tail and small concretions appear in the ascending excretory tubules in

the body. The flame cell number increases to 4 pairs (fig. 17) with each flame cell marking the location of a flame cell group in the fully developed cercaria. With further development, the flame cells increase in number (fig. 18) and the thin walled excretory bladder forms by enlargement of the common tubule just posterior to the ventral sucker. The formation of the definitive excretory pore was not followed in detail, but a definite evagination of the bladder toward the dorsal body wall was observed in immature cercariae at the site of the pore (fig. 19).

The pre-adult in the definitive host (figs. 20 to 25). The growth and development of *H. chelydrae* were observed in material from experimentally infected turtles, mostly *Chelydrae serpentina,* sacrificed at intervals up to 3 months after exposure. Until the 5th day, the worms differed little in size or structure from the body of the cercaria (fig. 20). They were extremely active and were found mostly in the terminal respiratory passages, but some migrated into the tissues and even through the surface of the lung after its removal from the host. Usually most of the internal structure of the parasite was obscured by the intestinal ceca distended with the blood of the host, but the basic pattern of the cercarial body was still evident. The excretory tubules which formerly served the caudal flame cells of the cercaria were curved medially and anteriorly close to the posterior end of the worm to end blindly at the level of the excretory bladder. At 7 days, some of the parasites were noticeably larger and more rounded than the cercarial body.

A snapping turtle infected 13 days previous to examination yielded 65 young worms that measured 0.6 to 0.7 by 0.4 to 0.5; cuticular spines were still present, but the excretory pore was distinctly more anterior, and the bladder more elongate than in the cercaria. The genital primordia had grown to 0.080 to 0.096 by 0.032 to 0.040 as measured in stained specimens, but was little if any further differentiated than in cercariae. The ventral sucker, however, was less distinct and appeared less muscular (fig. 21). Almost identical specimens were obtained from a *Chrysemys picta marginata* which had been infected for 17 days.

In a 21-day infection of *C. serpentina,* the 7 worms recovered and measured alive were up to 1.0 long and 0.3 to 0.5 wide (fig. 22). Cuticular spines had disappeared and the ventral sucker was not evident in living specimens, but examination of sections revealed that its limiting membrane was still present although the sucker was almost devoid of muscle fibers. The worms were pyriform in shape with a very active neck region. The voluminous intestinal ceca extended almost to the end of the body and well posterior to the level of the ventral sucker. The genital primordium measured about 0.17 by 0.1 in living specimens, and in sectioned ones did not reveal any distinct differentiation. The excretory bladder showed a marked elongation with the excretory pore at its anterior end, opening at about the mid-level of the body. The common and ascending excretory canals were evident with the first entering the bladder ventrally close to its posterior end and bifurcating at about the level of the excretory pore. The ascending branches, filled with concretions, still extended to the level of the pharynx.

In a 43-day infection from a snapping turtle, the living parasites measured 1.4 by 0.7 at medium extension (fig. 23). The excretory bladder was more elongate than in younger worms; its pore was just posterior to the intestinal bifurcation. The bladder was approximately 0.8 long. The genital primordium showed considerable differentiation. A central mass, measuring about 0.35 by 0.14, showed some tubular development, probably the beginning of the uterus, but the ovary was not

yet distinct. Extending posteriorly from the mass, the testis could be seen in sections only as two bands of cells, one dorsomedian to each cecum.

A 62-day infection in *C. serpentina* yielded 25 worms up to 2.0 long and 0.4 wide when measured alive. They were very active and a few could be seen moving within the intact lung. The body of the parasites was occupied largely by the distended intestinal ceca and no evidence of the ventral sucker remained. The excretory system (fig. 25) was essentially that of the adult trematode. The large, sac-like bladder extended from near the posterior end of the worm to the pharynx; the excretory pore opened at or slightly anterior to the level of the intestinal bifurcation. The common excretory canal blended into the ventral side of the bladder at about its midlevel, bifurcated about midway between that point and the excretory pore, while the ascending branches still extended to the pharyngeal level and contained conspicuous excretory concretions. The extent to which the reproductive system had developed is shown in figure 24. Although distinct, the gonads were not yet functional. The pair of narrow cords extending along the dorso-median borders of the ceca for about one-half their length had united to form the testis, an inverted U-shaped organ well posterior to the intestinal bifurcation. From the apex of the testis a band of cells extended anteroventrally to join the tube leading to the genital atrium and pore on the ventral surface just posterior to the oral sucker. The cirrus sac and seminal vesicle were not differentiated.

The ovary was rounded and located just posterior to the intestinal bifurcation and ventral to the excretory bladder. The oviduct extended a short distance from the posterior border of the ovary to receive the common vitelline duct. The vitellaria consisted of narrow bands of cells extending along the ventro-median border of the intestinal crura, united anteriorly to form the short common vitelline duct. The seminal receptacle and Mehlis' gland were not differentiated from the female gonoduct which continued as the uterus in the form of a loop in the dorsal body region between the ceca and extending well toward their ends. A short distance posterior to the ovary, the arms of the loop crossed, the ascending one extending anteroventrally more or less parallel to the male duct to reach the genital atrium.

A large snapping turtle examined 94 days after cercariae were expelled into the trachea yielded 32 *H. chelydrae* which measured 7.0 to 8.0 in length and contained many eggs with developing embryos but no miracidia. It was not certain that the worms were from the experimental infection, but the large number and uniform size and development strongly indicated that they were from that source. The preceding experiment thus shows that *H. chelydrae* evidently becomes ovigerous in large snapping turtles in less than 90 days in the laboratory. In the field, however, natural infections are acquired during the summer and do not normally produce fully developed miracidia until the following spring or summer. As indicated above, trematodes containing infective miracidia are not found until June. Since at least 20 days are required for development of the cercariae in the snail host, turtles could become naturally infected no earlier than July in north-central Indiana. In view of the fact that they begin "hibernation" in the fall, there would not be sufficient time for the parasite to mature before the host enters winter torpor. All experimental infections of turtles were made late in the summer and maintained in the laboratory at temperatures which were much higher than in the field where the parasites probably would develop at a much slower rate.

The rate at which the adult of *H. chelydrae* develops is probably affected also by the host species. Of the turtles used, *C. serpentina* seems to be the most favorable; worms recovered from a 62-day infection of that species were larger and further developed than those in a 76-day infection of a painted turtle, *C. picta,* and two 82-day infections in the false map turtle, *G. pseudogeographica.* Crowding did not seem to be a factor because the snapping turtle, although slightly larger than the others, yielded more parasites. Furthermore, the 2 map turtles were about the same size, but one harbored 16 worms and the other 32, all indistinguishable in size and structure.

The development of *H. chelydrae* in the definitive host is of particular interest because it explains the location of the excretory pore which is unique among the digenetic trematodes. Its dorsal location far toward the anterior end of the adult suggests that the pore might be a secondary one and that the definitive pore of the cercaria disappears. Such is not the case, however, as the above observations show. The pore is dorsal, but not far anterior, in a number of trematodes, among them the paramphistomes and certain opistholebetids, in which it is slightly anterior to the ventral sucker at the posterior end of such worms. The situation in *H. chelydrae* is a matter of differential growth which assumes various forms in the Digenea. Whereas in most trematodes post-cercarial growth is greatest in the hind body which contains the reproductive organs, in *Opistholebes* the body elongates between the suckers and changes from distomate to amphistomate in form. In *Moreauia,* on the other hand, growth is mostly lateral, so that the adult is several times as wide as long. In *H. chelydrae,* in which the adult may be 30 times as long as the cercarial body, the distance from the excretory pore to the anterior end remains fairly constant (figs. 20 to 23) with most of the elongation of the body due to growth in the ventral region. The common excretory canal leaving the anterior end of the bladder in the cercaria is thereby shifted until it and the bladder form a U followed by elongation of the posterior end of the bladder with the common canal blending into its ventral surface.

Heronimus chelydrae is unique also in the disappearance of a powerful ventral sucker as the parasite develops in the definitive host. As a rule, if a cercaria has a well developed ventral sucker, it remains so in the adult, often becoming a powerful adhesive organ. In certain eucotylids and cyclocoelids, it is poorly developed in the cercaria and persists in a degenerate state in the adult; whereas, in most microphallids and opisthorchioids, the ventral sucker is either lacking in the cercaria or very rudimentary and attains its full development in the metacercaria or adult. Certain monostomes, however, lack that sucker at all stages. In *H. chelydrae,* it is obviously useful in the migration of the cercarial body to the lungs; its disappearance thereafter may be an adaptation to the sheltered nature of that habitat. Lung flukes, in general, regardless of affinity, have poorly developed suckers, as do certain other adult trematodes that live elsewhere than in the digestive tract; e.g., the renicolids in the kidneys of birds.

Taxonomic Position of Heronimus chelydrae

In a preliminary note (Cable and Crandall, 1956), *H. chelydrae* was allocated to the superfamily Paramphistomatoidea on the basis of the cercaria and the embryology of its excretory system. La Rue (1957) accepted that arrangement in placing the family Heronimidae along with several other amphistome and monostome fami-

lies in the superfamily Paramphistomatoidea which, with the Notocotyloidea, comprises the suborder Paramphistomata. He proposed the order Echinostomida to include that suborder and the Echinostomata. That arrangement is based largely on larval stages and is essentially the one Szidat (1939) proposed, using Fasciolatoidea as the order name. From adult structure alone, the affinity of *H. chelydrae* to that group probably never would have been surmised.

Another feature that may give clues to affinity among the digenetic trematodes is the method of germinal multiplication in larval generations, as suggested by Cort, Ameel and Van der Woude (1954). They combined a limited reproductive potential of larval stages with other features in considering the paramphistomes to be a primitive group, and the echinostomes with the development of germinal masses as somewhat more specialized. On that basis, the life cycle of *H. chelydrae* is in closer agreement with the paramphistomes than the echinostomes. Although no exact counts of cercariae were made in this study, the number produced by one zygote probably is even less than that reported for certain amphistomes (225 for *Cotylophoron cotylophorum,* 180 for *Paramphistomum cervi*).

The excretory system of the cercaria and its embryology, which provide the basis for major groups in La Rue's scheme, are very similar in the paramphistomes and echinostomes, as Hussey (1941) has pointed out. In both groups, the bladder is non-epithelial, the excretory system is stenostomate, and the primary pores are in the tail. The principal differences concern the location of the primary and definitive excretory pores of the cercaria. In paramphistomes, the primary pores are near the tip of the tail and the definitive pore appears later on the dorsal surface near the posterior end of the body. In the echinostomes, the primary pores are on the sides of the tail not far from the body and the definitive pore is ventral in the body-tail furrow. Its dorsal location in the cercaria of *H. chelydrae* and the position of the primary pores near the tip of the tail further substantiate allocation of the Heronimidae to the superfamily Paramphistomatoidea rather than to the Echinostomatoidea.

There is abundant evidence for the validity of the family Heronimidae even though at present it contains but a single species, *H. chelydrae.* In addition to the adult features on which the family was originally based, the events of the life cycle and morphology of the cercaria differ from those of any other family to a degree that is not encountered in recognized families of digenetic trematodes. In the first place, the production of cercaria directly by the mother sporocyst distinguishes *H. chelydrae* from the echinostomes and paramphistomes, both typically with 2 redial generations, and occurs elsewhere as far as known only in the gasterostomes and perhaps certain brachylaemids. The possibility that the sac enclosing germinal elements in the miracidium of *H. chelydrae* may represent a degenerate larval generation has been mentioned above.

Unlike paramphistomes, the cercaria of *H. chelydrae* lacks eye-spots, the sucker is not at the extreme posterior end of the body, and the tail contains a pair of flame cell groups. Furthermore, the cercaria neither leaves the snail host nor encysts. The absence of eye-spots probably is of little significance and may be an adaptation to the failure of the larvae to emerge from the snail. As to the position of the ventral sucker, it is almost as far posterior in certain psilostome larvae as in the cercaria of *H. chelydrae;* indeed, some adult paramphistomes do not have it at the extreme posterior end of the body. The significance of flame cells in the basal

enlargement of the cercarial tail in *H. chelydrae* is uncertain. Elsewhere, they are so located in many but not all furcocercous larvae (sensu lato) and the developing cystophorous larva of *Halipegus*. In many furcocercous forms, the entire body and tail are spinose, whereas spines occur only on the body and basal enlargement of the tail in the cercaria of *H. chelydrae*. That distribution suggests that the basal enlargement could correspond to the posterior end of the body in other cercariae, an interpretation that would bring all the flame cells into the body region. There is no evidence, however, supporting such an interpretation in the embryology of the cercaria. On the other hand, the presence of flame cells in the tail may be due to a closer affinity between the Paramphistomatoidea and Strigeatoidea than has been suspected. Furthermore, the large cells in the basal swelling of the tail resemble the so-called caudal bodies of certain fork-tailed cercariae. Failure of cercariae of *H. chelydrae* to encyst has its counterpart in the life histories of certain strigeatoids including some strigeids, the blood flukes, brachylaemids, and gymnophalline fellodistomatids.

For a species whose cercaria does not leave the molluscan host, *H. chelydrae* is unusual in that the tail is an efficient swimming organ. As a rule, in larvae which do not emerge, the tail is very weak, reduced, or even lacking; such is the case also in many species which do emerge. The tail probably is not essential to completion of the life cycle in *H. chelydrae,* for it is quickly lost when the larvae fasten to the lining of the mouth or digestive tract of the definitive host and commence creeping. Turtles feeding on infected snails under water, however, probably crack the shell with their jaws and so can free a certain number of cercariae. If those released into the water were unable to swim, the chances of their again reaching the mouth of the turtle would be reduced. Even so, a high percentage of failures to reach the lungs of the turtle seems certain from the small number of worms that are usually found in natural infections.

SUMMARY

The life history of *H. chelydrae* has been determined experimentally and its stages described. The miracidium penetrates the snail *Physa integra* and develops into a branched sporocyst which produces amphistomate cercariae, directly, in about 20 days. Infections in the snail usually terminate within 60 days. Cercariae do not emerge or encyst; turtles acquire the parasite by ingesting infected snails. Cercariae released in the mouth or upper digestive tract migrate to the lungs by way of the trachea. In pre-adult growth, the ventral surface elongates, shifting the excretory pore to its position in the adult. The ventral sucker degenerates and eggs appear in the uterus by the 90th day. Eggs are not laid, but hatch in utero when the worm escapes and reaches water. The infection is seasonal, gravid worms occurring mostly during June and July in north-central Indiana. It is concluded that *H. chelydrae* shows closer affinities to the paramphistomes than any other trematode group, but differs in respects that support the validity of the family Heronimidae. The allocation of that family to the superfamily Paramphistomatoidea is reaffirmed.

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EXPLANATION OF PLATES

All figures drawn by microprojection of stained specimens with details added free-hand unless otherwise indicated.

Abbreviations: EC, excretory concretion; EP, excretory pore; EV, excretory vesicle; G, oral glands; GP, genital primordium; LG, lateral ganglion; OV, ovary; PH, pharynx; T, testis; UT, uterus; V, vitellaria; VS, ventral sucker.

EXPLANATION OF PLATE I

FIGURE 1. Miracidium showing arrangement of germinal elements.
FIGURE 2. Two-day sporocyst.
FIGURE 3. Seven-day sporocyst.
FIGURE 4. Twelve-day sporocyst showing excretory system.
FIGURE 5. Eleven-day sporocyst showing beginning of lateral branches.
FIGURE 6. Thirteen-day sporocyst.
FIGURE 7. Twenty-day sporocyst.
FIGURE 8. Forty-day sporocyst with germinal elements almost exhausted.

EXPLANATION OF PLATE II

FIGURE 9. Cercaria in dorsal aspect, showing flame cells of one side.
FIGURE 10. Cercarial body in dorsal aspect enlarged to show details of excretory system.
FIGURE 11. Sagittal section of the cercarial body.
FIGURES 12–19. Embryology of the cercarial excretory system. (Drawn free-hand from living material.)

EXPLANATION OF PLATE III

FIGURE 20. Three-day pre-adult in dorsal aspect, showing flame cells of left side.
FIGURE 21. Thirteen-day pre-adult in dorsal aspect, showing bladder and excretory pore.
FIGURE 22. Same, 22 days of age.
FIGURE 23. Same, 42 days of age.
FIGURE 24. Sixty-two-day worm in dorsal aspect, showing reproductive organs.
FIGURE 25. Same, showing bladder and main excretory canals.

RESEARCH NOTE

A TEST OF THE RESISTANCE OF *TAENIA SAGINATA* EGGS TO FREEZING.

In February 1957, several gravid proglottids and some free eggs of *Taenia saginata* in saline solution were received through the courtesy of Dr. Donald V. Moore, University of Texas, Dallas, Texas. The material had been in transit for about 2 days. After its arrival at Beltsville, it was stored at 45 F for about 36 hours. Then, because it was deemed insufficient for a test of survival of the eggs under natural conditions and had been stored in Dr. Moore's refrigerator at a mean temperature of about 24 F for about 4.5 days prior to shipment, it was utilized as follows: The free eggs were transferred to a small flask; the segments were dissected, and the eggs recovered from them by screening and washing were added to those in the flask. In this way about 900,000 brown-shelled eggs, plus many others, were obtained in 150 ml of water. From them, 3 aliquots, each containing about 300,000 brown-shelled eggs in about 50 ml of water, were prepared. One was immediately administered to a bovine animal. The others were stored in a refrigerator at a mean temperature of about 24 F; each was kept there until a few hours before it was administered to a bovine animal. The other essential data of the test are given in the following table:

Aliquot No.	Total exposure of eggs of *Taenia saginata* at 24 F (days)	Animal No.	Interval from administration of aliquot to slaughter of animal (days)	Degree of infestation with *Cysticercus bovis* cysts at necropsy
1	4.5	1	64	Fairly heavy; many cysts in heart and well distributed in skeletal muscles.
2	12.3	2	117	Moderate; fairly large number of cysts in heart and skeletal muscles.
3	76.1	3	60	Only two cysts found*

* Entire heart, masseter muscles, and diaphragm were finely sliced with a knife; remainder of carcass was inspected by making numerous incisions in musculature for examination of cut surfaces.

The results of the test are considered to show that under the conditions specified a considerable proportion of the *T. saginata* eggs withstood exposure to a temperature of about 24 F for about 2 weeks, and that a small proportion withstood such exposure for about 11 weeks. Penfold, Penfold, and Phillips (1936, J. Helm. **14**: 135–140) have reported that eggs kept in saline at 2 to 5 C (35.6 to 39 F) were not viable after 16.5 weeks.—JOHN T. LUCKER, *Animal Disease and Parasite Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland.*

PLATE I

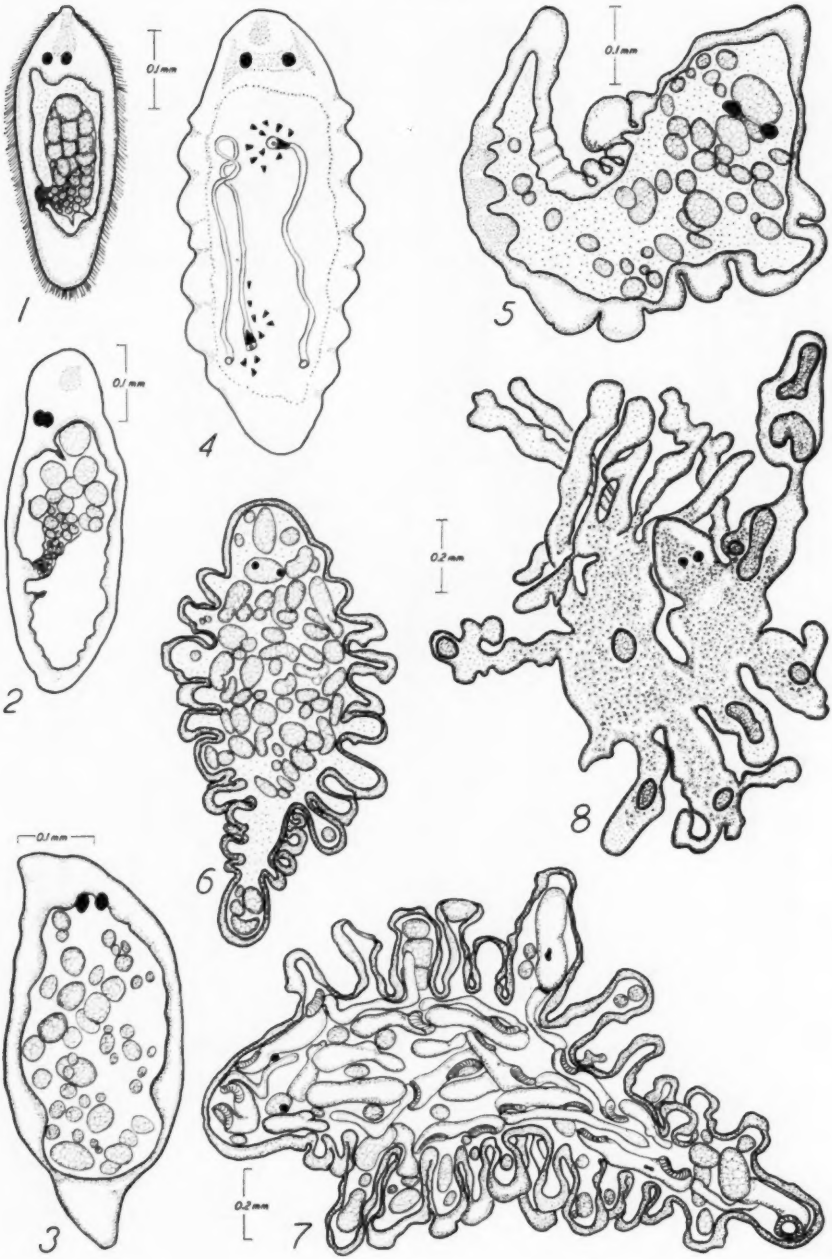


PLATE II

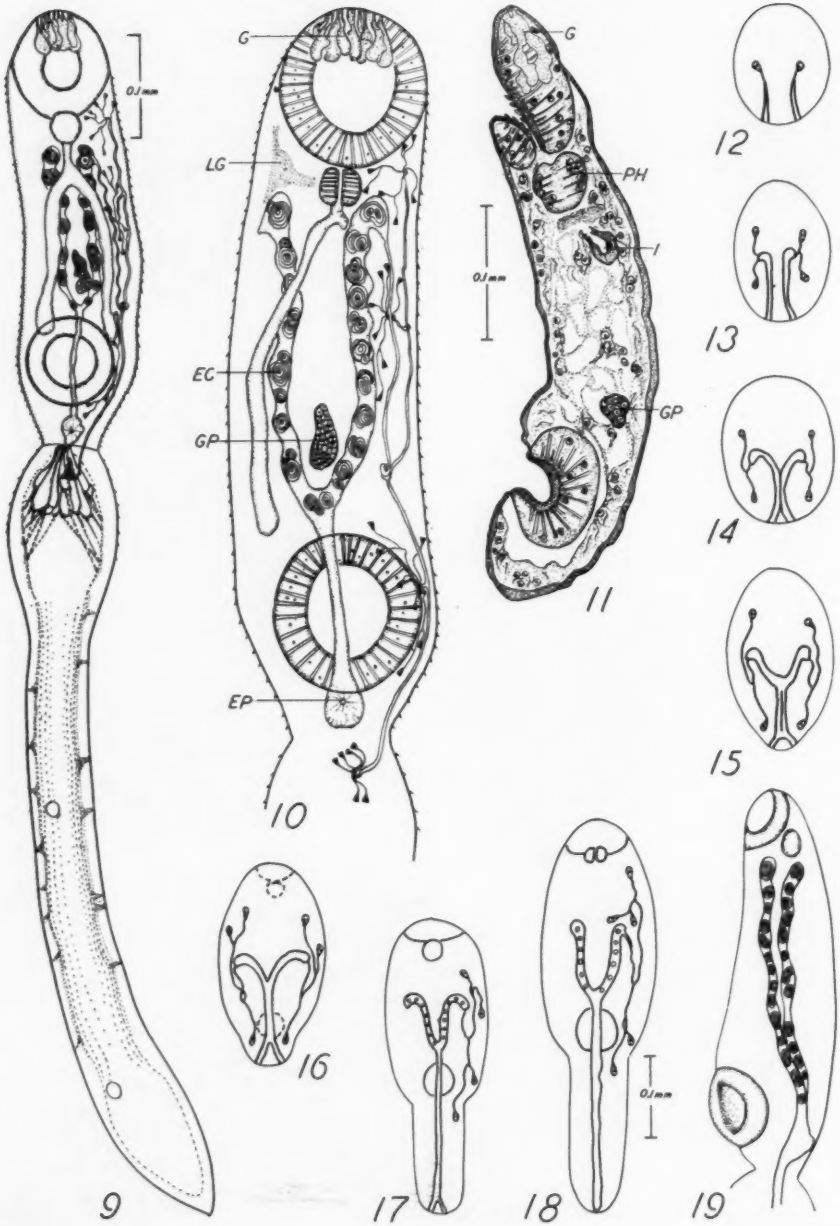
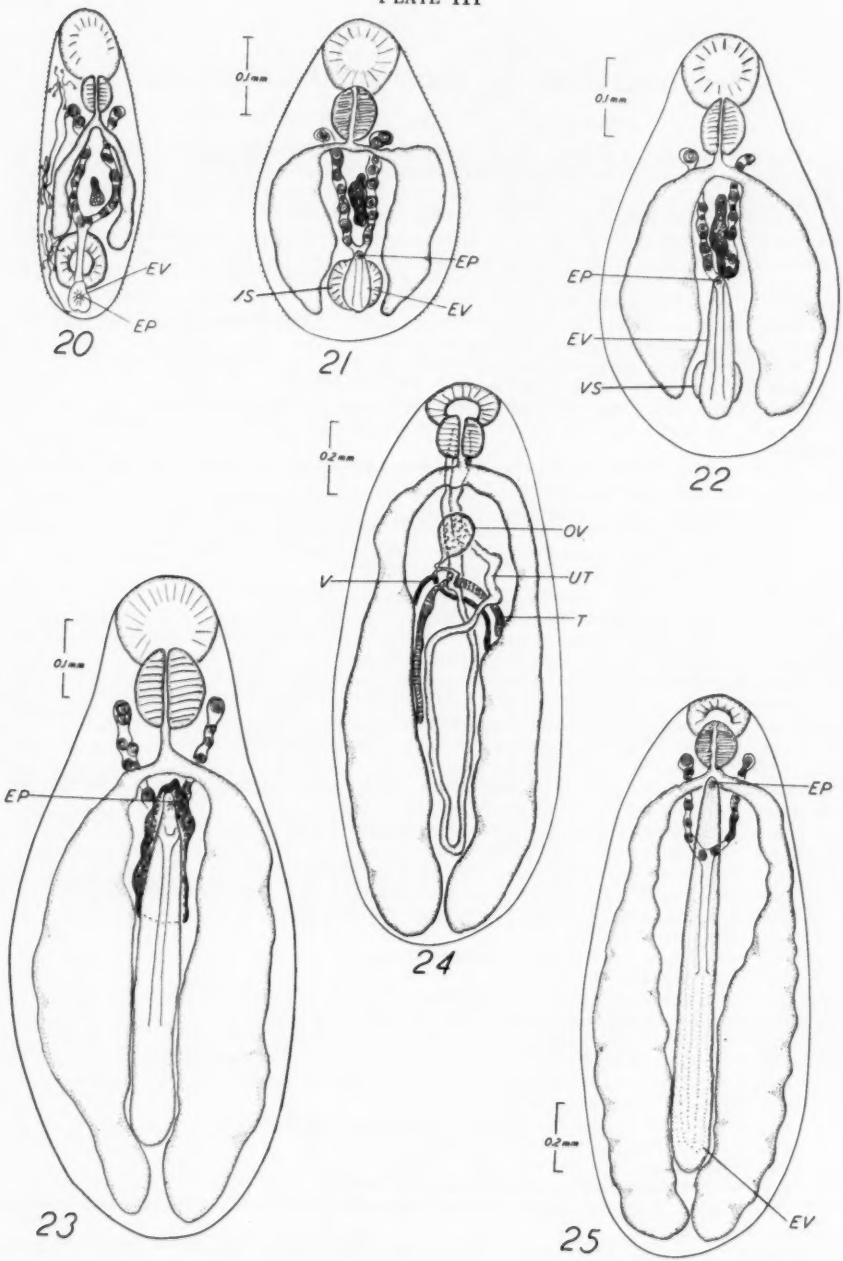


PLATE III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SUGGESTIONS TO PROSPECTIVE AUTHORS

II. ABBREVIATIONS FOR NAMES OF SERIALS.

The following list is believed to be representative of abbreviations for serials containing papers frequently cited in the Journal of Parasitology. Journal names of a single word are not abbreviated and for this reason do not appear in the list; e.g., Parasitology. Words of five letters or less are not abbreviated. Rare or exotic words are spelled out. The article is dropped when it is the first word in the name of a serial. German and French adjectives are capitalized as in the Index-Catalog of Medical and Veterinary Zoology, United States Department of Agriculture. Abbreviations of serials not appearing in this list can usually be found in the aforementioned Index-Catalog, though not always readily.

Acta Vet. Acad. Sci. Hung.: Acta Veterinaria Academiae Scientiarum Hungaricae
Am. J. Hyg.: American Journal of Hygiene
Am. J. Pub. Hlth.: American Journal of Public Health
Am. J. Trop. Med.: American Journal of Tropical Medicine
Am. J. Trop. Med. Hyg.: American Journal of Tropical Medicine and Hygiene
Am. J. Vet. Med.: American Journal of Veterinary Medicine (*continued as* Veterinary Medicine)
Am. J. Vet. Res.: American Journal of Veterinary Research
Am. Midl. Nat.: American Midland Naturalist
Am. Mus. Nov.: American Museum Novitates
An. Inst. Biol.: Anales del Instituto de Biologia. Mexico, D.F.
An. Inst. Med. Trop.: Anais do Instituto de Medicina Tropical. Lisbon
Anat. Rec.: Anatomical Record
Ann. Inst. Past., Paris: Annales de l'Institut Pasteur. Paris
Ann. Parasit.: Annales de Parasitologie Humaine et Comparée
Ann. Soc. Belg. Med. Trop.: Annales de la Société Belge de Médicine Tropicale
Ann. Trop. Med. Parasit.: Annals of Tropical Medicine and Parasitology
Arch. Inst. Past., Afr. N.: Archives des Instituts Pasteur de l'Afrique du Nord
Arch. Inst. Past., Alg.: Archives de l'Institut Pasteur d'Institut Pasteur d'Algérie
Arch. Inst. Past., Tunis: Archives de l'Institut Pasteur de Tunis
Arch. Ital. Sci. Med. Colon.: Archivio Italiano di Sienze Mediche Coloniali
Arch. Prot.: Archiv für Protistenkunde
Arch. Schiffs- u. Tropen-Hyg.: Archiv für Schiffs- und Tropen-Hygiene, Pathologie, und Therapie Exotischer Krankheiten (*discontinued in 1941*)
Arch. Wiss. Prakt. Tierh.: Archiv für Wissenschaftliche und Praktische Tierheilkunde
Arch. Zellforsch.: Archiv für Zellforschung
Arch. Zool. Expér. Gén.: Archives de Zoologie Expérimentale et Générale
Austral. J. Exp. Biol.: Australian Journal of Experimental Biology and Medical Science
Austral. J. Vet. Med.: Australian Journal of Veterinary Medicine
Avian Dis.: Avian Diseases
Biol. Abs.: Biological Abstracts. Philadelphia
Biol. Bull.: Biological Bulletin
Bol. Chil. Parasit.: Boletin Chileno de Parasitologica
Bull. Ent. Res.: Bulletin of Entomological Research. London
Bull. Inst. Past., Paris: Bulletin de l'Institut Pasteur. Paris
Bull. Soc. Path. Exot.: Bulletin de la Société de Pathologie Exotique. Paris
Can. J. Med. Sci.: Canadian Journal of Medical Science
Can. J. Microb.: Canadian Journal of Microbiology
Can. J. Res., Sec. D: Canadian Journal of Research, Section D
Can. J. Zool.: Canadian Journal of Zoology
Cbl.: Centralblatt (See Zbl., but always use as given)
Clin. Vet., Milano: La Clinica Veterinaria, etc. Milano
Compt. Rend. Acad. Sci., Paris: Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences. Paris
Compt. Rend. Soc. Biol.: Comptes Rendus des Séances de la Société de Biologie et de ses Filiales et Associées. Paris

(*Continued on p. 316*)

SOME LECITHODENDRIID METACERCARIAE FROM INDIANA AND MICHIGAN*

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In a review article on arthropods as intermediate hosts of worm parasites, M. C. Hall (1929) listed six members of the trematode family Lecithodendriidae as having their metacercarial stage in various aquatic insects, including members of the orders Diptera, Odonata, Plecoptera, Ephemeroptera, Trichoptera, and Coleoptera. Since that time, Brown (1933), Mathias and Vignaud (1935), Abdel Azim (1936), McMullen (1936), Okabe (1937), Knight and Pratt (1955), Okabe and Shibue (1951), and Macy and Moore (1954) have demonstrated that arthropods frequently if not always serve as the second intermediate hosts of members of this family.

The present paper gives additional evidence of that role by reporting several metacercariae from aquatic arthropods collected from rivers in Indiana and Michigan during an investigation of lecithodendriid life histories begun in 1955.

MATERIALS AND METHODS

A total of 343 aquatic arthropods included Hydracarina, 1; Amphipoda, 4; Decapoda, 6; Plecoptera, 12; Ephemeroptera, 47; Odonata, 200; Hemiptera, 4; Megaloptera, 25; Trichoptera, 15; Lepidoptera, 1; Coleoptera, 20; and Diptera, 8. Most of them were taken from the Wabash and Tippecanoe Rivers, Indiana, and their tributaries during the summer and fall of 1955, 1956, and 1957, but 26 were collected from the Manistee and Pere Marquette Rivers, Michigan, in September 1956.

Arthropods were examined by teasing with dissecting needles in 0.3 percent saline; some infections could be detected in intact hosts which were fairly transparent. Encysted metacercariae were removed from their cysts with dissecting needles or by the method of Macy and Moore (1954) employing weak concentrations of sodium hydroxide. Fixation was in hot 3 percent formalin, AFA, or corrosive sublimate-acetic acid, with or without cover glass pressure; specimens were stained with Semichon's aceto-carmine and prepared as whole mounts, or were sectioned and stained with hematoxylin and eosin. Measurements and drawings are based in part on living and in part on fixed and stained material; diagnoses are based chiefly on whole mounts fixed in hot corrosive sublimate-acetic acid. Figures were drawn free hand, free hand to scale, or with the aid of a microprojector as indicated; all measurements are in millimeters.

OBSERVATIONS

Most of the metacercariae found were not developed to the extent that they could be identified and were present in insufficient numbers for feeding experiments. Exceptions to that situation were the following 2 metacercariae, the first of which is progenetic in the arthropod host.

Neoprosthodendrium progeneticum n. g., n. sp.
(Figs. 1–3)

Generic diagnosis: Lecithodendriidae, Lecithodendriinae, Lecithodendriea. Body thick, little flattened, oval to pyriform in outline. Cuticle delicate and easily ruptured by pressure, with very

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* From a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Purdue University, August 1958.

fine spines seen only in living specimens under oil immersion lens. Ventral sucker pre-equatorial, smaller than oral sucker; prepharynx small or absent; esophagus and ceca short. Genital pore median or submedian, slightly anterior to ventral sucker. True cirrus sac absent; seminal vesicle, pars prostatica, ejaculatory duct, and prostatic cells contained in a thin condensation of parenchyma forming a false cirrus sac anterior to ventral sucker. With a mid-ventral depression anterior to false cirrus sac containing a papilla having no connection with gonoducts but possibly serving as an accessory copulatory structure in the absence of a cirrus. Testes symmetrical to slightly diagonal, posterior to ventral sucker. Vitellaria in forebody. Ovary dextral, immediately anterior to or overlapping right testis. Seminal receptacle and probably Laurer's canal present. Uterus confined to hindbody except ascending limb which passes dorsal to ventral sucker to join inconspicuous metraterm. Eggs rather large for the family. Excretory bladder U- to V-shaped.

Specific diagnosis: With the characters of the genus. Body 0.362 to 1.442 long, 0.247 to 1.003 wide. Oral sucker 0.084 to 0.182 long, 0.091 to 0.196 wide; mouth subterminal. Ventral sucker 0.064 to 0.140 long, 0.097 to 0.134 wide; average sucker ratio 1.19: 1.00. Pharynx cordiform to spherical, 0.043 to 0.078 long, 0.048 to 0.073 wide; esophagus narrow and sinuous; ceca voluminous, extending somewhat posterior to ventral sucker and filled with a substance which is green in living specimens, crystalline and eosinophilic after fixation. False cirrus sac small; seminal vesicle tubular and coiled, pars prostatica well developed, ejaculatory duct short. Testes equal or subequal, oval to pyriform, 0.250 to 0.364 long, 0.278 to 0.357 wide. Vitellaria lateral, consisting of 20 to 30 follicles on each side; follicles up to 0.070 in diameter, from level of oral sucker to that of ventral sucker. Ovary oval, entire, 0.178 to 0.406 long, 0.214 to 0.428 wide; usually overlapping both ventral sucker and right testis. Seminal receptacle between testes, spherical, 0.165 to 0.214 in diameter. Eggs numerous to very numerous, 0.035 to 0.046 long, 0.020 to 0.033 wide. Excretory bladder with wide arms nearly reaching testes.

Host: Naiad of the damselfly, *Hetaerina americana.*

Site: Hemocoel (not encysted).

Locality: Tippecanoe River, Indiana.

Type Specimens: Type and paratypes, U. S. National Museum Helminthological Collection, No. 39013.

The short ceca, U- to V-shaped excretory bladder, median and preacetabular position of the genital pore, and obscure body spination place *Neoprosthodendrium* in the Lecithodendriinae. The absence of a true cirrus sac and the proximity of the genital pore to the ventral sucker places it in the Tribe Lecithodendriea Skarbilovich, 1943. It resembles *Prosthodendrium* Dollfus, 1931, in the pretesticular position of the vitellaria, but is distinguished from that genus by having the testes situated behind the ventral sucker and from all genera of the tribe by the presence of the unique accessory papilla on the ventral surface of the body immediately anterior to the false cirrus sac.

All of 8 *Hetaerina americana* naiads collected from the Tippecanoe River during the summer of 1956 contained *N. progeneticum* in numbers ranging from 1 to 6 per insect. Of 6 naiads collected from the same locality in the summer of 1957, only 1 harbored that trematode while another contained 3 encysted metacercariae probably belonging to the Lecithodendriidae on the basis of body shape and size, short intestinal ceca, symmetrical arrangement of the primordia of the testes, and the V-shaped excretory bladder. One of these cysts showed thinning of the inner wall at one point as described elsewhere for metacercariae of *Mosesia chordeilesia.* Failure of the cercaria of that species to penetrate *Hetaerina americana,* however, indicates that the metacercariae from that insect were not those of *M. chordeilesia.* On the other hand, the thinning of the cyst wall suggests that the metacercaria could be that of *N. progeneticum* before escape from the cyst as postulated in the discussion of that stage of *M. chordeilesia* (Hall, 1959).

Progenesis has been reported previously for at least 3 lecithodendriids: for *Pleurogenes medians* by Sinitsin (1905), Mathias (1924), and Dollfus (1924); for *Pleurogenes japonicus* by Okabe and Shibue (1951); and for *Lecithodendrium*

(= *Prosthodendrium*) *chilostomum* by Skrjabin (1915). In the first 2 species, however, the metacercaria produces eggs while still enclosed in the cyst and does not wander free in the body cavity of the insect as does *N. progeneticum*.

Eumegacetes sp.
(Fig. 4)

Description: Body thick and muscular, 1.086 to 1.710 long, 0.510 to 0.823 wide, yellow in living specimens. With very fine spines which are embedded in the cuticle and usually not apparent in fixed specimens. Mouth subterminal; suckers powerful, subequal; oral sucker 0.178 to 0.528 long, 0.207 to 0.524 wide; ventral sucker postequatorial, 0.228 to 0.570 long, 0.257 to 0.620 wide; sucker ratio 0.67 to 1.08 : 1.00. Numerous cephalic glands with ducts in groups except at sides of oral sucker. Pharynx well developed, transversely oval, 0.078 to 0.185 long, 0.114 to 0.250 wide; esophagus short, frequently coiled under pharynx; intestinal bifurcation near pharynx, ceca long, narrow, extending almost to posterior extremity. Genital pore ventral, median, at pharyngeal level. Cirrus sac present, straight, containing tubular seminal vesicle and pars prostatica. Testes symmetrical, entire, round to oval, 0.071 to 0.121 long, 0.051 to 0.160 wide, situated about midway between ventral sucker and intestinal bifurcation; vasa efferentia short, extending almost medially to unite just before entering cirrus sac. Vitellaria not developed. Ovary entire, oval to spherical, median, 0.036 to 0.091 long, 0.043 to 0.082 wide, situated behind ventral sucker. Seminal receptacle small, immediately anterior or median to ovary; Laurer's canal probably present; Mehlis' gland situated to left of seminal receptacle. Uterus not apparent. Excretory vesicle dorsal, Y-shaped, with short stem and long arms which pass anteriorly to level of pharynx, giving off numerous lateral branches which extend to margin of body and sometimes curve ventrally before ending as blind, finger-like sacs. Entire vesicle of encysted specimens filled with small concretions which obscure the remainder of the excretory system.

Hosts: Naiads of *Gomphus* spp., *Dromogomphus* sp., *Ophiogomphus* (?) sp.

Site: Hemocoel (encysted or not).

Localities: Tippecanoe and Wabash Rivers, Indiana; Manistee River, Michigan.

Eumegacetes metacercariae were found in 17 of 34 *Gomphus* spp. naiads and in 11 of 32 *Dromogomphus* sp. naiads collected in Indiana during the summers of 1956 and 1957. One naiad taken from the Manistee River, Michigan, and tentatively identified as *Ophiogomphus* sp. also harbored these metacercariae while no parasites were found in 4 similar naiads collected from the Tippecanoe River. *Dromogomphus* never contained more than 2 metacercariae whereas 1 to 16 were found in *Gomphus*.

The worms were usually enclosed in a tough, hyaline, oval capsule apparently of parasite origin and measuring 1.2 to 1.6 long, 0.7 to 1.1 wide, and up to 0.115 in thickness. These cysts were loose in the hemocoel; occasionally the cyst wall was very thin or absent so that the worms were free in the body cavity.

In worms of the same size, there was sufficient difference in the size of the suckers and the position of internal organs with respect to the ventral sucker to suggest that the material represented more than one species of *Eumegacetes*. However, these differences did not correlate with ecological factors such as differences in host or locality, and specific differentiation among members of this genus has in the past depended chiefly on characters visible only in completely mature specimens. Attempts to bring the metacercariae to maturity by feeding them to newly hatched chicks were unsuccessful. However, Stafford (1931) obtained adults of *Eumegacetes medioximus* by feeding metacercariae from *Gomphus externus* and/or *Gomphus plagiatus* to chicks; failure in the present instance probably was due to an insufficient amount of material.

Several additional metacercariae were found in various aquatic arthropods. The worms were not sufficiently developed to identify them, even to genus, but as five

probably belong to the Lecithodendriidae, they are recorded below as an aid to further life history studies in that group.

Encysted Metacercariae in Stonefly Naiads

Metacercariae were found in the abdominal muscles of 4 to 12 stonefly naiads (*Pteronarcys* sp. and another undetermined genus); each infected insect harbored one to 10 cysts 0.106 to 0.200 in diameter and up to 0.014 in wall thickness. The body cuticle contains coarse spines which are readily visible in living and in fixed specimens. A stylet is present, either in the oral sucker or embedded in the cyst wall; it has a well-defined shoulder region, incurved edges of the shaft, and a total length (sclerotized portion only) of 0.016. The excretory bladder is V-shaped and filled with concretions.

Unencysted Metacercariae in Stonefly Naiads
(Fig. 5)

A few metacercariae were found free in the body cavities of 4 of 12 stonefly naiads (*Pteronarcys* sp. and unidentified specimens) from the Wabash and Tippecanoe Rivers. In fixed specimens, the body is elongate-oval, 0.260 to 0.349 long, and 0.121 to 0.168 wide. The cuticle is covered with fine spines which are best seen in living specimens. The sucker ratio is 1.13 to 1.54: 1.00 with the ventral sucker smaller and postequatorial. Other features are shown in figure 5. The intestine is filled with an orange-brown fluid in living specimens. The excretory vesicle may extend through one-fifth to over one-half of the body length.

Encysted Metacercariae in Crayfish
(Fig. 6)

Two of 6 unidentified crayfish were found to contain 1 and 30 metacercariae, respectively, encysted in the thoracic and abdominal muscles. When removed from the cyst, the body was flattened, oval to pyriform in outline, and measured 0.164 to 0.200 long, 0.121 to 0.135 wide in fixed specimens. The cuticle is covered with minute scales anteriorly and fine spines posteriorly. The excretory pore is situated on a papilla which may be withdrawn, leaving a small notch at the posterior end of the body. Other characteristics are shown in figure 6.

Encysted Metacercariae in Mayfly Naiads

A few encysted metacercariae resembling those of *Mosesia chordeilesia* as described in a previous paper (Hall, 1959) were found in the body cavities of 2 of 11 *Stenonema*(?) sp. and 8 of 27 *Hexagenia limbata* naiads. The cysts measure 0.128 to 0.207 in diameter with a primary wall thickness of 0.010 to 0.033. The cuticle is spinose anteriorly and a stylet with the sclerotized portion measuring 0.018 long is present, either in the oral sucker or embedded in the cyst wall. Because of insufficient material, feeding experiments were not attempted with the metacercariae, but the ones in *Hexagenia limbata* at least may well have been metacercariae of *M. chordeilesia.*

Unencysted Metacercariae in Mayfly Naiads
(Fig. 7)

Smaller numbers of unencysted metacercariae were found in 7 of 27 *Hexagenia*

limbata naiads collected from the Wabash and Tippecanoe Rivers during the summers of 1956 and 1957. The body is flattened, up to 0.264 long and 0.164 wide. Other features are shown in figure 7. In young metacercariae, the gonads are represented by only a C-shaped primordium, but in older ones they are differentiated and the fundament of the terminal structures is such as to suggest that the adult is a species with the genital pore lateral or even posterior to the ventral sucker.

Besides being unencysted, this species is distinguished from the metacercaria of *Mosesia chordeilesia* by the finer body spines embedded in the cuticle, the average sucker ratio (1.59 : 1.00), the more posterior location of the ventral sucker, and the location of the testes anterior to that sucker.

SUMMARY

Seven metacercariae, probably all different species, are reported as natural infections in aquatic arthropods collected from streams in Indiana and Michigan. One, which develops progenetically to full maturity free in the body cavity of *Hetaerina americana* (Odonata), is described as *Neoprosthodendrium progeneticum* n. g., n. sp. Another which occurs either encysted or free in the hemocoel of gomphid naiads belongs to the genus *Eumegacetes*.

ACKNOWLEDGMENTS

This study was supported by an XR Fellowship from the Purdue Research Foundation, 1956–1958, and has been supervised by Dr. R. M. Cable to whom the writer is indebted for counsel and criticism. He is indebted also to Dr. B. E. Montgomery, Department of Entomology, Purdue University, for the identification of some of the insect larvae.

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EXPLANATION OF PLATE I.

FIGURE 1. *Neoprosthodendrium progeneticum*, ventral view of whole mount. (Free hand to scale.)

FIGURE 2. Same, false cirrus sac, enlarged. (Free hand.)

FIGURE 3. Same, cross section through ventral surface anterior to false cirrus sac showing accessory papilla. (Drawn with a microprojector.)

FIGURE 4. *Eumegacetes* sp., ventral view of whole mount. (Drawn with a microprojector.)

FIGURE 5. Unencysted metacercaria from stonefly naiad, ventral view of whole mount. (Free hand to scale.)

FIGURE 6. Encysted metacercaria from crayfish, ventral view of whole mount removed from cyst. (Free hand to scale.)

FIGURE 7. Unencysted metacercaria from mayfly naiad, dorsal view of whole mount. (Free hand to scale.)

Abbreviations: AP, accessory papilla; EV, excretory vesicle; GO, genital opening; IN, intestinal cecum; MT, metraterm; OS, oral sucker; OV, ovary; PP, pars prostatica; SR, seminal receptacle; SV, seminal vesicle; TE, testis; UT, uterus; VI, vitellaria; VS, ventral sucker.

RESEARCH NOTE

BRACHYLAIME MICROTI FROM THE DEER MOUSE IN UTAH.

Brachylaime microti Kruidenier and Gallichio, 1959 (Tr. Am. Micr. Soc. **78**: 428) was described from specimens found in the pancreatic duct of the long-tailed meadow mouse *Microtus longicaudus baileyi* Goldman taken in the Grand Canyon National Park, Coconino County, Arizona. In August 1957, specimens of this same trematode were found by one of us (J. C. F.) in the fine bile ducts within the liver of 3 specimens of the deer mouse *Peromyscus maniculatus sonoriensis* (Le Conte) collected at Trout Creek Canyon, Deep Creek Mountains, Juab County, Utah, elevation 6,100 feet. This is a desert trapsite situated on the extreme west central boundary of Utah. The biota of the trapsite corresponds to that characteristic of the Upper Sonoran life zone of Merriam. Kruidenier and Gallicchio apparently obtained their mice in a much more moist habitat ("Swamp Lake"). Only 1 of our specimens of *B. microti* was obtained undamaged. It had evidently migrated into the small intestine, from which it was removed alive.

Species of *Brachylaime* have not heretofore been reported from the Great Basin or from cricetine rodents west of the Mississippi River. Prior to the present report, flukes of this genus have apparently not been recovered from the bile ducts within the liver of their host.

Since 1950, we have examined many hundreds of cricetid and heteromyid rodents from the Lake Bonneville Basin of Utah and have failed to find other brachylaimids. The focus of infection appears to be restricted to the eastern slopes of the Deep Creek Mountains, Utah. It is hoped that collections of land snails from this focus will enable us to elucidate the life cycle of *B. microti*.

In their paper describing *B. microti*, Kruidenier and Gallicchio included an addendum (1935–1956) to the list of *Brachylaime* species compiled by Dollfus (1935, Ann. Parasit. **13**: 52). Omitted from this addendum was *B. oesophagei* Shaldybin, 1954 (Rabot. Gel'mintol. 75-Let. Skrjabin, 747) from *Sorex araneus, S. minutus,* and *Neomys fodiens* taken in the Mordow A. S. S. R. (European Russia). *B. tisa* Chatterji, 1958 (Indian J. Helm. **8**: 92), from the kite *Butastur tisa* taken in Orissa, India, has been described since the publication of Kruidenier and Gallicchio.

We wish to express our appreciation to Mr. Allen McIntosh, U. S. National Museum, for comparing our specimens with the holotype of *B. microti*.

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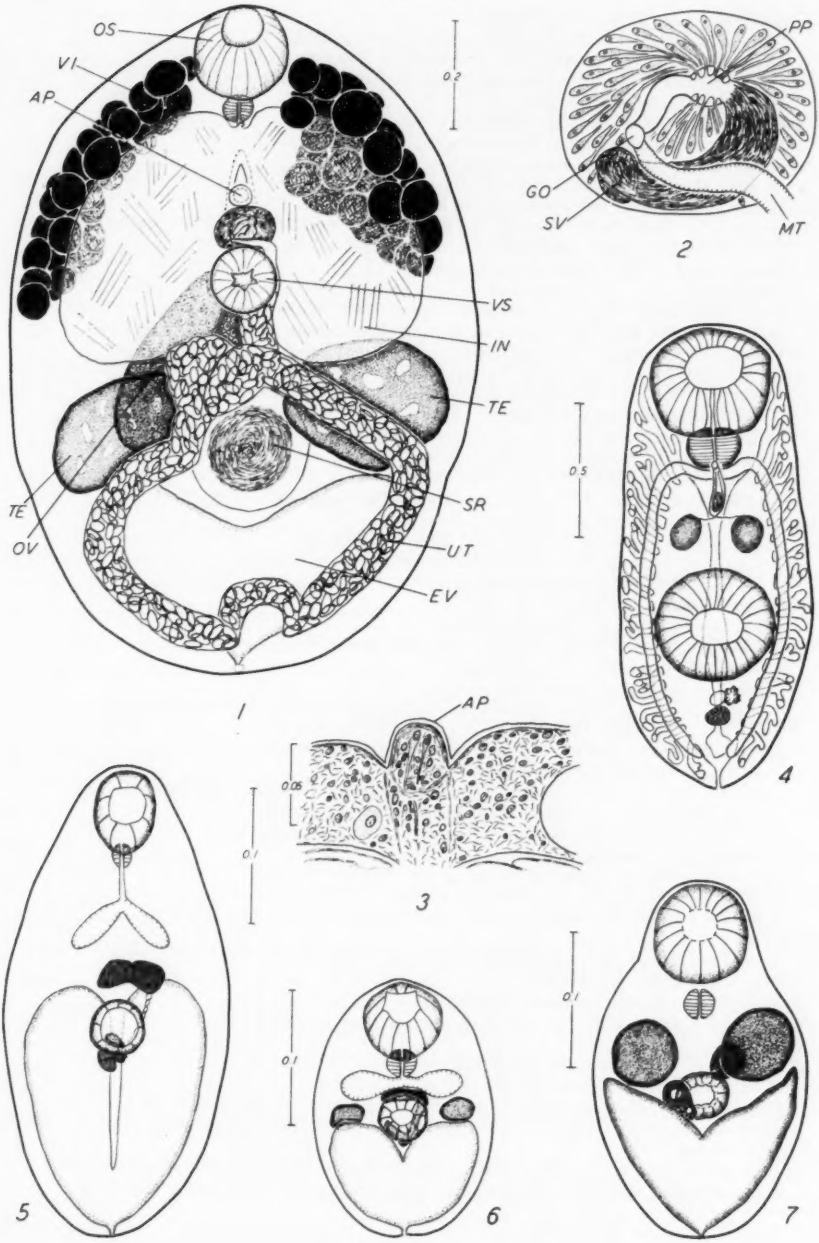
PLATE I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(*Continued from p. 308*)

Cor. Vet.: The Cornell Veterinarian
Deut. Med. Wschr.: Deutsche Medizinische Wochenschrift
Deut. Tierärztl. Wschr. Deutsche Tierärztliche Wochenschrift
Deut. Tropenmed. Ztschr.: Deutsche Tropenmedizinische Zeitschrift
Diss. Abs.: Dissertation Abstracts
Ecol. Monog.: Ecological Monographs
Exp. Parasit.: Experimental Parasitology
Helm. Abs.: Helminthological Abstracts
Ill. Biol. Monog.: Illinois Biological Monographs
Indian J. Med. Res.: Indiana Journal of Medical Research
Indian J. Vet. Sci.: Indian Journal of Veterinary Science and Animal Husbandry
Indian Med. Res. Mem.: Indian Medical Research Memoirs
Indian Vet. J.: The Indian Veterinary Journal
Iowa State Coll. J. Sci.: Iowa State College Journal of Science
Jap. J. Exp. Med.: Japanese Journal of Experimental Medicine
Jap. J. Med. Sci. Biol.: Japanese Journal of Medical Science and Biology. Tokyo
J. Agr. Res.: Journal of Agricultural Research, U. S. Department of Agriculture
J. Am. Med. As.: Journal of the American Medical Association
J. Am. Vet. Med. As.: Journal of the American Veterinary Medical Association
J. Bact.: Journal of Bacteriology
J. Biol. Bd. Can.: Journal of the Biological Board of Canada
J. Biol. Chem.: Journal of Biological Chemistry
J. Clin. Path.: Journal of Clinical Pathology. London
J. Comp. Path. Ther.: Journal of Comparative Pathology and Therapeutics
J. Econ. Ent.: Journal of Economic Entomology
J. Exp. Biol.: Journal of Experimental Biology. London
J. Exp. Med.: Journal of Experimental Medicine
J. Helm.: Journal of Helminthology. London
J. Hyg.: Journal of Hygiene. London
J. Inf. Dis.: Journal of Infectious Diseases
J. Lab. Clin. Med.: Journal of Laboratory and Clinical Medicine
J. Mammal.: Journal of Mammalogy
J. Morph.: Journal of Morphology
J. Natl. Mal. Soc.: Journal of the National Malaria Society (*discontinued in 1951*)
J. Parasit.: Journal of Parasitology
J. Path. and Bact.: Journal of Pathology and Bacteriology
J. Prot.: Journal of Protozoology
J. S. Afr. Vet. Med. As.: Journal of the South African Veterinary Medical Association
J. Trop. Med. Hyg.: Journal of Tropical Medicine and Hygiene
J. Wash. Acad. Sci.: Journal of the Washington Academy of Sciences
J. Wildl. Mgt.: Journal of Wildlife Management
Med. Abs. J.: Medical Abstracts Journal (in Russian)
Med. J. Austral.: Medical Journal of Australia
Med. Parazitol. i Parazitar. Bolezni: Meditsinskaia I'arazitologiia i Parazitarnye Bolezni. Moskva
Mem. Inst. Osw. Cruz: Memórias do Instituto Oswaldo Cruz
Mil. Med.: Military Medicine
N. Am. Vet.: North American Veterinarian
Natl. Inst. Hlth. Bull. No. —: National Institute of Health Bulletin No. —. Washington, D. C.
Phil. J. Sci.: Philippine Journal of Science
Poul. Sci.: Poultry Science
Proc. Am. Vet. Med. As.: Proceedings of the American Veterinary Medical Association
Proc. 4th Internatl. Cong. Zool.: Proceedings of the Fourth International Congress of Zoology. Cambridge
Proc. Helm. Soc. Wash.: Proceedings of the Helminthological Society of Washington
Proc. Soc. Exp. Biol. Med.: Proceedings of the Society for Experimental Biology and Medicine
Proc. U. S. Live Stock San. As.: Proceedings of the United States Live Stock Sanitary Association

(*Continued on p. 326*)

STUDIES ON A MICROSPORIDIAN HYPERPARASITE OF STRIGEOID TREMATODES. I. PREVALENCE AND EFFECT ON THE PARASITIZED LARVAL TREMATODES.*

WILLIAM W. CORT, KATHLEEN L. HUSSEY AND DONALD J. AMEEL

The sporocysts and cercariae of a large proportion of the strigeoids in the snails of the area around the University of Michigan Biological Station have hyperinfections with a microsporidian of the genus *Nosema*. The literature on microsporidian parasites of trematodes was summarized by Dollfus (1946). *Nosema légeri* Dollfus, 1912, and *N. spelotremae* Guyénot, Naville and Ponse, 1925, are parasites of metacercariae in marine bivalves; *Glugea danilewskyi* Guyénot, Naville and Ponse, 1925, and *G. encyclometrae* Guyénot and Naville, 1922, were in adult trematodes of snakes and *N. distomi* Lutz and Splendore, 1908, was described as a hyperparasite of an unidentified adult trematode in *Bufo marinus* in Brazil. The only description we have found in the literature of a microsporidian in trematode cercariae in snails was by Brumpt (1922, p. 336) who reported the finding of a *Nosema* species, which he named *N. echinostomi*, in the rediae, cercariae and metacercariae of an echinostome in *Limnaea limnosa* in the forest of Boulogne near Paris. Further description of *N. echinostomi* was given by Dollfus (1946) from unpublished data given him by Brumpt. Adult echinostomes that developed from experimental infections in ducks did not harbor this microsporidian. The infection developed slowly in the rediae and cercariae in the snail host. When the spores were not very numerous in the cercariae they could develop into metacercariae, but in heavy infections the cercariae were killed. The rediae containing the spores lost their usual yellow color and became white; if they were only slightly parasitized they produced some cercariae, which were themselves parasitized. Brumpt noted that tetracotyles of strigeoids that were found in the hyperparasitized echinostome rediae were not infected. The spores of *N. echinostomi* are ellipsoidal with a length of about 5 microns, and resemble the spores described for other species of the genus *Nosema*. Brumpt did not attempt to describe the stages in detail or to work out the life cycle of this species.

The information in the literature on the microsporidian hyperparasites of trematodes is rather confusing on account of the lack of agreement between the different investigators on their classification and on the descriptions of the stages in their life cycles. We have found no recent investigations on microsporidian parasites of trematodes. However, Martin (1936) published a brief note on a "sporozoan" found in larval trematodes in *Succinea* sp. in the region of Woods Hole, Massachusetts. It was present both in the germinal sacs and emerged cercariae of 3 trema-

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tode species, and also in the metacercariae of one of them, an echinostome, which encysted in the snail host. The author stated that "cysts" of this hyperparasite of various sizes were present both in the snail's tissues and in the larval trematodes. This parasite produced considerable injury to the larval trematodes and even the emerged cercariae were quite abnormal. Martin suggested that it was a normal parasite of the snail that had become adapted to living in the larval trematodes.

There are a number of reports in the literature of microsporidian hyperparasites of cestodes (Döllfus, 1946). Recently, Dissanaike (1957 a, b) has published the results of investigations on *Nosema helminthorum* Moniez, 1887, a hyperparasite of cestodes, which he found in *Moniezia* spp. in England and in Pakistan.

Our interest in the microsporidian in the strigeoids of the snails in the Douglas Lake region has been primarily in its host-parasite relations as a hyperparasite of trematodes. We have not been able as yet to work out in sufficient detail the stages of its life cycle, especially those in schizogony, to make adequate comparisons with the described species of the genus *Nosema*. For that reason we will not at this time give it a specific name, but will refer to it as *Nosema* sp.

OBSERVATIONS

Infections with *Nosema* sp. were first seen many years ago at the Michigan Biological Station in larval strigeoids, in which the daughter sporocysts and cercarial embryos were greatly distorted. Year after year infections of this type were noted, but nothing was ever done with them, and the cause of the distortion was not understood until the summer of 1955.

Nosema sp. up to the present time has been found only in the region around the University of Michigan Biological Station in the northern tip of the southern peninsula of Michigan. In this area it is a hyperparasite in the large majority of strigeoid infections in the snails. Snails harboring *Nosema* have been collected from the shores of small inland lakes (Douglas Lake and Crooked Lake), from one place on the shore of Lake Huron, and from small ponds and streams. Most of the collections were from sandy beaches, but some of the snails came from swampy areas on the lakes or from the muddy bottoms of ponds or streams. We have no evidence that the ecological environment of the snail host affected the incidence of the *Nosema* sp. in the larval strigeoids.

The procedure in examining a collection of snails for microsporidian infection was first to pick out strigeoid infections from which cercariae were escaping by separating the individual snails into bottles; the bodies of the snails with mature strigeoid infections were then removed from their shells and the sporocysts and developing stages of cercariae were examined with the high power of the microscope for the stages of *Nosema* sp. Then examinations were made of as many as possible of the snails from which no cercariae had escaped, in order to include immature strigeoid infections and very heavy *Nosema* infections in which the strigeoid cercariae were so badly injured that very few or none were escaping.

During 3 summers, 1956–1958, *Nosema* sp. has been found in the larval stages of 12 different species of strigeoid trematodes, viz, (1) *Diplostomum flexicaudum* (Cort and Brooks, 1928) in *Stagnicola emarginata angulata* (Sowerby), *S. emarginata canadensis* (Sowerby), *Lymnaea stagnalis appressa* Say, *L. stagnalis perampla* Walker and *Fossaria abrussa* (Say); (2) *Cotylurus flabelliformis* (Faust, 1918) in *L. stagnalis appressa* Say and *L. stagnalis perampla* Walker; (3) *Posthodiplo-*

stomum minimum (MacCallum, 1921) (*C. physae* Cort and Brooks, 1928) in *Physa parkeri* Currier, *P. magnalacustris* Baker and *Physa* sp.; (4) *Uvulifer ambloplites* (Hughes, 1927) (*C. bessiae* Cort and Brooks, 1928) in *Helisoma trivolvis* (Say) and *H. campanulatum smithii* (Baker); (5) *Apatemon* sp. (*Cercaria burti* Miller, 1923) in *Physa* sp. and *H. campanulatum smithii* Baker; (6) *Cercaria laruei* Cort and Brooks, 1928, in *S. emarginata angulata* (Sowerby); (7) *Cercaria yogena* Cort and Brackett, 1937, in *S. emarginata angulata* (Sowerby); (8) *Cercaria dohema* Cort and Brackett, 1937, in *L. stagnalis appressa* Say, *L. stagnalis perampla* Walker; (9) *Cercaria emarginatae* Cort, 1917, in *S. emarginata angulata* (Sowerby); (10) *Cercaria modicella* Cort and Brooks, 1928, in *Fossaria abrussa* (Say); and (11, 12) two unidentified species of strigeoids, one in *S. emarginata angulata* (Sowerby) and the other in *H. trivolvis* (Say) and *H. campanulatum smithii* Baker.

In only a very few of the hyperinfections with *Nosema* sp. were spores present. Many of the others contained stages that were readily recognized as belonging to sporogony. However, a large proportion of the infections were identified by the presence of stages which we have learned to recognize as belonging to *Nosema* sp. without understanding their detailed structure or their exact place in the life cycle. These stages appear to belong to schizogony since the stages of sporogony are not difficult to follow.

The spores of *Nosema* sp. are typical of the genus. They are oval to pear-shaped with a clear space at the larger end (fig. 1, a and b). Measurements of 50 spores from several different infections varied from 3.9 to 6 microns by 2.2 to 3.8 microns and had an average of 4.9 by 3 microns. It was possible sometimes to produce extrusion of the filament (fig. 1, c), which in one case measured over 100 microns.

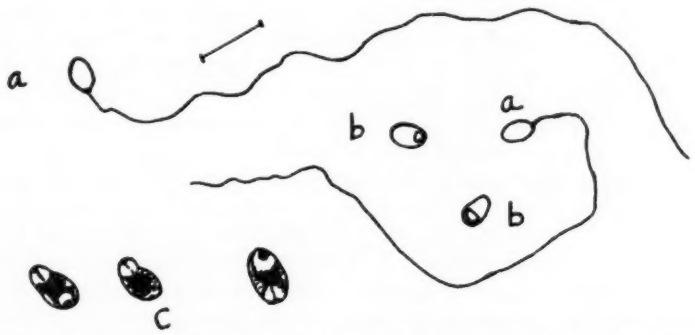

FIGURE 1. *Nosema* sp. a and b drawn with the aid of the camera lucida. Scale = 0.01 mm. a, spore with extruded filament; b, fresh spore; c, spores from sections stained with iron hematoxylin. Free hand drawing.

The mature spores were commonly found in protuberances on surfaces of mother and daughter sporocysts and cercarial embryos of all stages (pl. I, figs. 3, 6, 8, 9; pl. II, figs. 3, 5, 7, 8). These protuberances varied from those containing only 1 spore to very large ones with many spores. The spores frequently were freed during examination (pl. I, fig. 2). Sometimes the protuberances appeared to be solid masses of spores, but frequently they contained a large proportion of fluid

along with the spores (pl. I, fig. 9). Sometimes the spores could be seen on the surface just under the cuticle, especially of small cercarial embryos, with practically no protuberances (pl. II, fig. 3). Development of spores can also occur on the inner surface of the body wall of both mother and daughter sporocysts and spores are almost always found free in their body cavities. In very heavy spore-producing infections practically all the tissues of the larval trematodes are replaced by spores. We have not yet worked out the detailed cytology of the stages of development in sporogony, but they seem to be similar to the description given by Dissanaike (1957b) for this stage in *N. helminthorum*.

We have no information on the emergence of the sporoplasm or how it reaches and penetrates into the larval strigeoids, nor have we ever recognized any stage of *Nosema* sp. in the tissues of the snail. We have not yet been able to work out the cytological stages of schizogony, but the earliest stages (probably belonging to schizogony) which we recognize in living material as belonging to this microsporidian are small vesicles which can be best seen in small cercarial embryos (pl. I, fig. 5; pl. II, fig. 5). These vesicles contain granules and structures which appear to be the degenerating nuclei of host cells. What may also be stages of schizogony are rounded masses made up of many equal sized small bodies, which are sometimes seen attached to the surface of the inner wall of sporocysts.

Infections of *Nosema* sp. which contain fully developed spores are rarely seen. In the 3 summers during which extensive examinations have been made for this microsporidian, spores have been found in only about 30 strigeoid infections, including *D. flexicaudum, P. minimum* (*C. physae*), *U. ambloplites* (*C. bessiae*), *C. emarginatae, C. modicella* and the species from *Helisoma* which we have not been able to identify. It seems probable that spore-producing infections of *Nosema* sp. could occur in any species of strigeoid infected with this microsporidian. The severity of the spore-producing infections with *Nosema* has varied from one in which only 1 infected daughter sporocyst was found to one in which all the daughter sporocysts and cercarial embryos were dead and disintegrating with almost all of their tissues replaced by fully developed spores. However, in most of the spore-producing hyperinfections almost all of the daughter sporocysts were infected and few if any normal cercariae were being produced.

In the largest percentage of the *Nosema* infections examined only vesicles or vesicles and a few developing spores were present. Such infections can be best identified by the examination of numbers of small cercarial embryos that have been broken out of the daughter sporocysts. These are listed as light *Nosema* infections. Others with larger numbers of daughter sporocysts affected, and more of the external protuberances (stages of sporogony) on the cercarial embryos are considered as moderate infections. The few heavy infections, with most or all of the daughter sporocysts affected, included some with mature spores and those in which no spores could be found. The numbers of examinations of snails infected with the larval stages of strigeoids for hyperinfections of *Nosema* sp. is small, except for *Diplostomum flexicaudum*. We will, therefore, give only data on this species to illustrate the extent of *Nosema* sp. infection in the Douglas Lake region. Of 137 snails (*S. emarginata, L. stagnalis* and *F. abrussa*) with *D. flexicaudum* infection from a series of collections from the shores of Douglas Lake, 20 were listed as negative, 90 as lightly infected, 20 as having moderate infections and 7 as heavy. In all except the heavy infections large numbers of normal cercariae were being produced.

The extent of the injury that may be produced in daughter sporocysts by infection with *Nosema* sp. is clearly shown by comparing normal and infected sporocysts (pl. I, fig. 4). Distinguishing characteristics of badly infected sporocysts are greatly increased size, opacity, and loss of motility. In some instances only a part of the sporocyst may be affected and contain embryos loaded with spores, while its remaining portions may be full of normal embryos. In infected sporocysts the body wall may be at least 3 times its normal thickness. In a few cases we found infected mother sporocysts which were still producing daughters containing all stages of *Nosema* infection. Since we only rarely find mother sporocysts in mature strigeoid infections, we do not know how commonly they are hyperinfected. Surface protuberances of various sizes with different stages of sporogony are commonly seen on daughter sporocysts (pl. I, fig. 3; pl. II, fig. 8). Vesicles like those so commonly seen in cercarial embryos were not noted in sporocysts, but there were frequently present rounded masses attached to the inner surface of the wall which we interpreted as stages of schizogony. Different stages of *Nosema* sp. were found in germinal masses, in daughter sporocysts in various stages of development, and in cercarial embryos of different sizes (pl. II, figs. 2, 3, 6).

One or more elements of a germinal mass may be almost entirely replaced by developing spores (pl. II, fig. 2). Round and oval cercarial embryos may contain only small vesicles, or be bloated and distorted with later stages (pl. II, fig. 6). Fully developed spores may be present on very young cercarial embryos. In embryos with only the beginning of tail development, there may be only small vesicles, but also not infrequently such embryos may show bloating and distortion of the body and tail (pl. I, figs. 1, 5, 8). These small embryos may also be covered with extensive protuberances containing all stages of sporogony including mature spores. Larger cercarial embryos have all stages of *Nosema* infection varying from a few vesicles to almost complete replacement of their tissues by spores (pl. I, figs. 6, 9; pl. II, figs. 3, 5, 6, 7). Even very badly distorted embryos were still alive and capable of movement. Tissue destruction may be quite extensive and the cercarial embryos very much bloated. For example, in one small cercarial embryo 6 flame cells were observed whose tublues had been destroyed and whose "flames" were beating freely in the spaces.

Nosema sp. differs from the "sporozoan" described by Martin (1936) in the absence of hyperinfection in the fully developed cercariae that were able to escape naturally from the snail host. In very extensive series of examinations of emerged cercariae, including 638 from a spore-producing infection, only 1 was found in which stages of *Nosema* sp. could be recognized. It appears, therefore, that the presence of stages of *Nosema* in well developed cercarial embryos prevents the completion of development and emergence. Frequently the larger cercarial embryos in infected sporocysts are very distorted and grotesque in appearance (pl. I, fig. 7; pl. II, fig. 4).

Probably the most remarkable distortion of the cercarial embryos of *D. flexicaudum* by *Nosema* infection was in those cases where there was a beginning of precocious development of metacercariae with the retention of the cercarial tails. In such specimens the bodies were greatly distorted, bloated, and even covered with spores with the still attached tails capable of considerable movement. In a few infections of *D. flexicaudum* the daughter sporocysts contained only precociously developed metacercariae in which stages of *Nosema* sp. were present (Olivier, 1940).

We observed a few snails, especially specimens of *L. stagnalis*, in which the metacercariae (tetracotyles) of *C. flabelliformis* were infected with *Nosema* sp. Such infections were also produced experimentally and will be described in the second paper of this series.

DISCUSSION

From the location of the spores of *Nosema* sp. in the mother sporocysts and in the strigeoids in the digestive gland of the snail host, it can be suggested that they can only be freed by the death and disintegration of the snail hosts. This release could be accomplished either by the natural death of the snails or by the ingestion of them by birds or other vertebrates; it seems very probable that the spores could survive passage through the digestive tract of vertebrates. It is difficult also to imagine any other method of entrance of the spores of *Nosema* into the snails except by ingestion. This entrance has been demonstrated in a large series of experiments which will be reported in the second paper of this series. Although the incidence of spore-producing infections is surprisingly low, the numbers of spores produced in each is so great that they would be spread widely in the environment. Probably most of the snails feeding on the bottom would ingest only a few spores, but occasionally snails might obtain large numbers. If the snails ingesting the spores had immature strigeoid infections, the hyperinfection of the mother sporocysts and the daughters which had not yet left the mother might produce infection in a large proportion of the daughters in the digestive gland. If the spores were ingested after completion of migration of the daughter sporocysts into the digestive gland, they could only be infected by direct penetration of the sporoplasms released from the spores. It also seems probable that the sporoplasms can only be released in the digestive tract of the snail and that they must migrate through the snails' tissues to reach the strigeoid sporocysts. We have no evidence of *Nosema* infection in snail tissue. Therefore, infection of a large proportion of the daughter sporocysts in the snail's digestive gland might be accomplished either by the ingestion of unusually large numbers of spores or the infection of daughters while still within the mother.

The majority of infections we have encountered in the Douglas Lake area have been those in which only a few daughter sporocysts were hyperinfected with only the early stages of *Nosema*. With the information now available it is difficult to understand why there is such a large proportion of light infections of *Nosema* sp. in which only early stages are present with no production of spores.

We have no evidence that stages of *Nosema* sp. can pass from one daughter sporocyst to another in the digestive gland of the snail. However, almost all of our experimental infections have been spore-producing, with the completion of development in 2 to 3 weeks. In all of them the snails received very large numbers of spores. It, therefore, might be suggested that the large proportion of natural infections with only a small proportion of the daughter sporocysts containing only early stages of the microsporidian is due to the development of a resistance of the strigeoids against the microsporidian and also its failure to pass from one daughter sporocyst to another.

SUMMARY

A large proportion of the strigeoid infections in the snails in the area surrounding the University of Michigan Biological Station is hyperinfected with a species of *Nosema*. Only a small proportion of the hyperinfections are spore-producing.

Mother and daughter sporocysts and cercarial embryos of all stages contain the stages of this microsporidian. Emerged cercariae are free of infection because large cercarial embryos when infected are so injured and distorted that they cannot escape from the snail. The details of cytological structure of the stages in the life cycle of this microsporidian have not yet been worked out. Early stages of schizogony appear as small vesicles, which can best be seen in cercarial embryos, and the stages of sporogony in protuberances on the surfaces of sporocysts and cercarial embryos appear to be like those of other species of *Nosema*. So far this hyperparasite has been found only in the Douglas Lake area at the northern tip of the southern peninsula of Michigan. Examinations have shown that 12 different species of strigeoids in the area harbor this infection. While heavy infections may kill the larval trematodes, in most cases the injury is slight and does not greatly decrease cercarial production.

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Explanation of Plates

Plate I

Figure 1. Very young cercarial embryo of *Apatemon* sp. (*C. burti*) showing small vesicles and protuberances.

Figure 2. Cercarial embryos of *Apatemon* sp. (*C. burti*) filled with spores.

Figure 3. Entire young daughter sporocyst of *Posthodiplostomum minimum* (*C. physae*) showing effects of the infection.

Figure 4. Normal and infected strigeoid daughter sporocyst.

Figure 5. Young cercarial embryo showing vesicles.

Figure 6. Cercarial embryo showing bloating and masses of spores.

Figure 7. Normal and infected cercarial embryos of *Diplostomum flexicaudum*.

Figure 8. Cercarial embryo of *Diplostomum flexicaudum* with surface protuberances and spores.

Figure 9. Same as figure 8 under high power.

Plate II

Figure 1. Cercarial embryo of *D. flexicaudum* containing numerous vesicles which produce localized bloating.

Figure 2. Germinal mass from daughter sporocyst of *Apatemon* sp. (*C. burti*) with one hyperinfected element.

Figure 3. Very young cercarial embryo of *D. flexicaudum* with spores on its surface.

Figure 4. Normal and infected cercarial embryos of *D. flexicaudum*.

Figure 5. Cercarial embryo of *D. flexicaudum* showing some vesicles and surface protuberances.

Figure 6. Young oval cercarial embryo of *P. minimum* (*C. physae*) showing bloating.

Figure 7. Cercarial embryo of *D. flexicaudum* containing enormous numbers of spores.

Figure 8. Anterior tip of daughter sporocyst of *D. flexicaudum* with protrusions produced by *Nosema* infection.

PLATE I

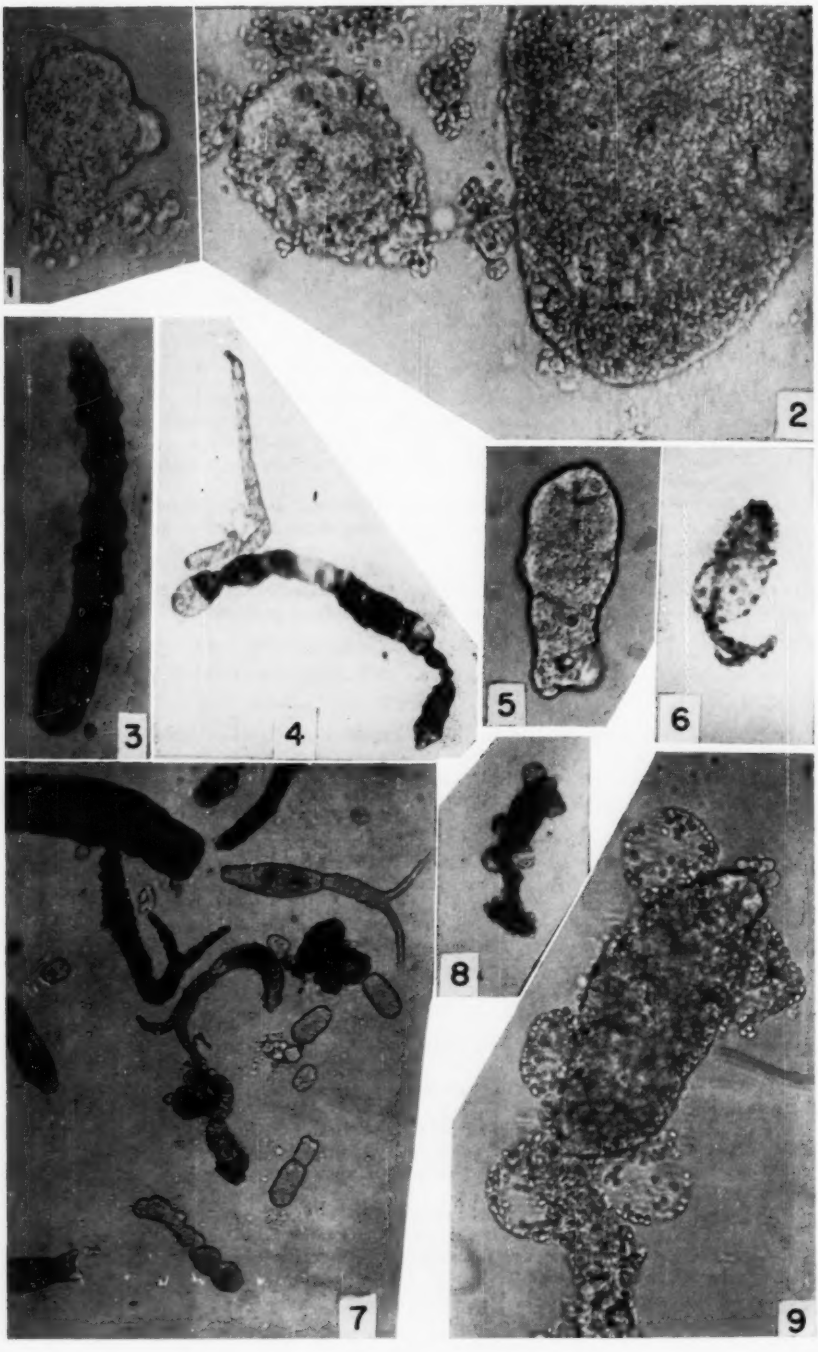
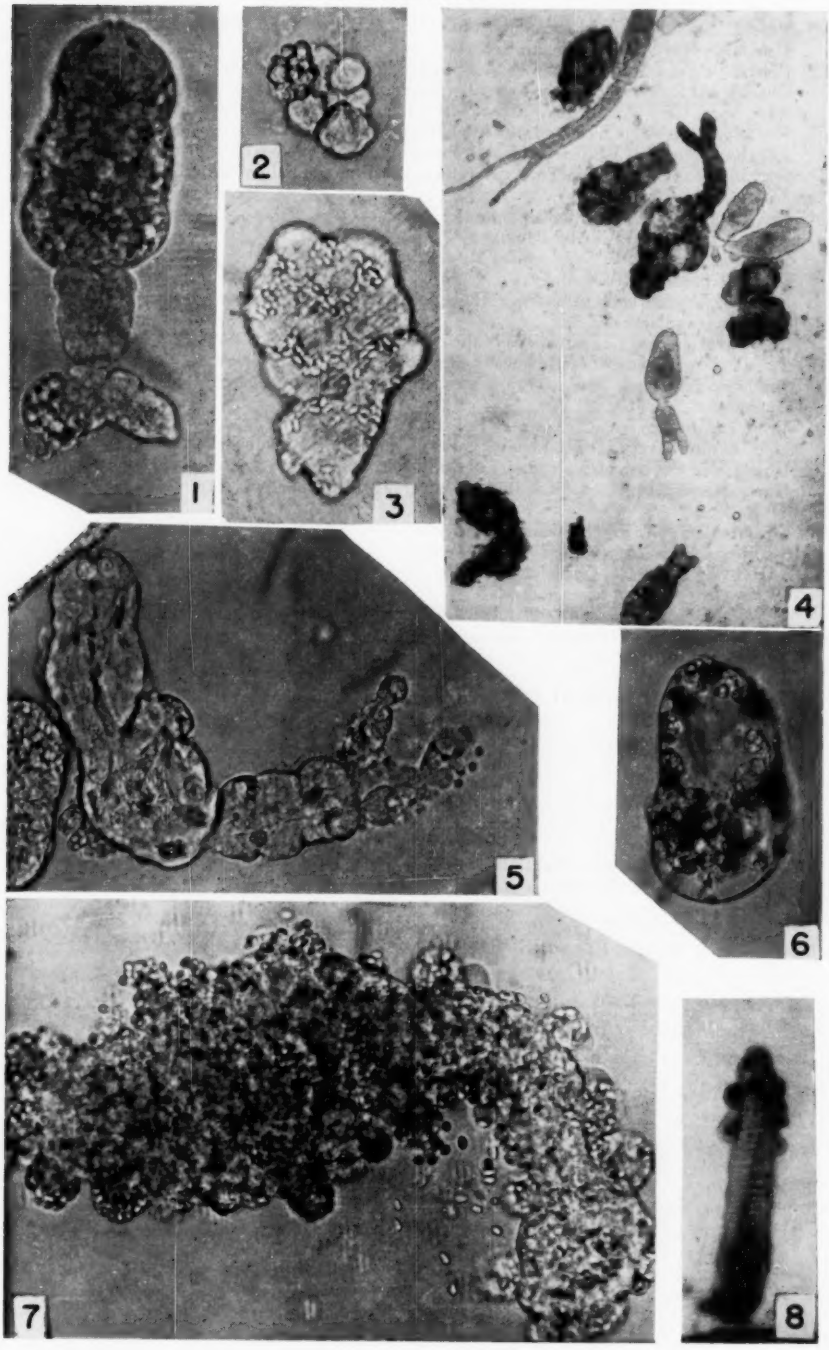


PLATE II



(*Continued from p. 316*)

Proc. U. S. Natl. Mus.: Proceedings of the United States National Museum
Pub. Hlth. Rept.: Public Health Reports
Pub. Hlth. Serv. Publ.: Public Health Service Publication
Quar. J. Micr. Sci.: Quarterly Journal of Microscopical Science
Rept. Surg.-Gen. U. S. Army: Report of the Surgeon-General of the United States Army
Rev. Appl. Ent., Ser. B: Review of Applied Entomology, Series B. London
Rev. Ibér. Parasit.: Revista Ibérica de Parasitologiá
Rev. Inst. Salub. y Enferm Trop.: Revista del Instituto de Salubridad y Enfermedades Tropicales. México, D. F.
Riv. Parassit.: Rivista di Parassitologia. Rome
S. Med. J.: Southern Medical Journal
Smithson. Misc. Coll.: Smithsonian Miscellaneous Collection
Stain Tech.: Stain Technology
Stanford Univ. Publ., Univ. Ser., Biol. Sci.: Stanford University Publications, University Series, Biological Sciences
Tr. Am. Micr. Soc.: Transactions of the American Microscopical Society
Tr. Kans. Acad. Sci.: Transactions of the Kansas Academy of Science
Tr. Roy. Soc. Trop. Med. Hyg.: Transactions of the Royal Society of Tropical Medicine and Hygiene
Trop. Dis. Bull.: Tropical Diseases Bulletin. London
U. S. Armed Forces Med. J.: United States Armed Forces Medical Journal
Univ. Calif. Publ. Zool.: University of California Publications in Zoology
Univ. Minn. Agr. Exp. Sta. Bull.: University of Minnesota Agricultural Experimental Station Bulletin
Vet. Med.: Veterinary Medicine
Vet. Rec.: Veterinary Record
WHO Chron.: WHO Chronicle
Wildl. Dis.: Wildlife Disease
Zbl. Bakt. 1. Orig.: Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. I. Abteilung. Originale. Jena
Zbl. Bakt. 1. Ref.: Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. I. Abteilung. Referate. Jena
Zbl. Bakt. 2.: Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten. II. Abteilung. Jena
Zool. Anz.: Zoologischer Anzeiger
Zool. Jahrb. Abt. Syst.: Zoologische Jahrbücher. Abteilung für Systematik, etc. Jena
Ztschr. Hyg.: Zeitschrift für Hygiene
Ztschr. Hyg. u. Infkr.: Zeitschrift für Hygiene und Infektionskrankheiten. Liepzig, Berlin
Ztschr. Parasitenk., Berl.: Zeitschrift für Parasitenkunde (Zeitschrift fur Wissenschaftliche Biologie, Abt. F). Berlin
Ztschr. Parasitenk., Jena: Zeitschrift für Parasitenkunde. Jena
Ztschr. Tropenmed. Parasit.: Zeitschrift für Tropenmedizin und Parasitologie
Ztschr. Vergl. Physiol.: Zeitschrift für Vergleichende Physiologie
Ztschr. Wiss. Zool.: Zeitschrift für Wissenschaftliche Zoologie. Leipzig

—ELERY R. BECKER, *Editor*

STUDIES ON A MICROSPORIDIAN HYPERPARASITE OF STRIGEOID TREMATODES. II. EXPERIMENTAL TRANSMISSION*

WILLIAM W. CORT, KATHLEEN L. HUSSEY AND DONALD J. AMEEL

In the first paper of this series (Cort, Hussey and Ameel, 1960) we reported on investigations made during the summers of 1956, 1957, and 1958 on the prevalence and effect on the parasitized larval trematodes of a microsporidian hyperparasite of the genus *Nosema*, which is found in a large proportion of the strigeoid infections in the snails of the region around the University of Michigan Biological Station near the northern tip of the southern peninsula of Michigan. We also carried out series of experiments designed to solve some of the problems involved in the transmission of this species of *Nosema*. As in the first paper of this series, we will refer to this microsporidian hyperparasite as *Nosema* sp. and will not try to determine its exact specific relationships until more details of its life cycle have been worked out.

In a preliminary experiment done during the summer of 1956 we tried to find out what happened to the spores of *Nosema* that were ingested by snails that harbored no trematode infections. Several small laboratory raised juveniles of *Physa parkeri* were placed in small dishes with enormous numbers of the spores of *Nosema* sp. They were seen to ingest the spores during the period they were in the dishes with them, so that very large numbers must have entered their digestive tracts. Examinations were made of 2 of these snails, one after 36 hours and the other after 72 hours. Although their tissues were carefully teased apart and examined under the high power of the microscope, no trace of the spores or of anything recognizable as developmental stages of the microsporidian was found. Two more of the snails were killed and fixed for sectioning, one at 36 and the other at 72 hours after exposure to the spores. In examination under high power of the microscope of sections of the whole bodies of these snails no trace of the spores or of anything recognizable as a stage of *Nosema* sp. was found.

Rather late in the summer of 1956 an experiment was performed in which snails harboring larval strigeoids were exposed to very large numbers of the spores of *Nosema* sp. During the summer of 1957 four more experiments of this same type were carried out, in which it was determined how long it took experimental infections to produce mature spores. In one of these experiments it was demonstrated that the metacercariae (tetracotyles) of *Cotylurus flabelliformis* could be infected with *Nosema* sp. Two further experiments in the summer of 1958 showed that the spores could survive at least a year and that several different species of larval strigeoids could be hyperinfected experimentally with this microsporidian.

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In all these experiments the snails were permitted to ingest large numbers of the *Nosema* spores. A difficulty in planning the experiments was that we were not able to obtain in the area around the University of Michigan Biological Station snails harboring strigeoids that were entirely free from hyperinfections with *Nosema* sp. Experimental infections of snails with strigeoids are so difficult to carry out and take such a long time, that we were forced to use snails which we knew from previous examinations had natural hyperinfections of *Nosema* sp.

RESULTS

Experiment I

On August 10, 1956, we had available in the laboratory 10 adults of *Stagnicola emarginata angulata* that had mature infections of *Diplostomum flexicaudum*. They came from a beach on Douglas Lake where a large proportion of the snails with strigeoid infections had natural hyperinfections with *Nosema* sp.

On August 10 we exposed 5 of these snails to large numbers of *Nosema* spores from a very heavy natural infection and kept the other 5 as controls. The snails placed in dishes with the *Nosema* spores were seen to ingest the spores during the whole time they were with them. All these snails lived and were examined 11 days after exposure to the spores.

Two of the controls had very little if any natural hyperinfection with *Nosema* sp. in the larval stages of *D. flexicaudum* which they harbored. In 2 other control snails a small proportion of the cercarial embryos had early stages of *Nosema* sp. However, the fifth control snail had a very heavy *Nosema* infection, with most of the sporocysts and cercarial embryos of its *D. flexicaudum* infection loaded with spores.

All of the 5 snails which had been exposed to the *Nosema* spores had heavy hyperinfections in their strigeoids. A large proportion of the daughter sporocysts in all of them were enlarged, discolored, and had protuberances on their surfaces. All but a few of the cercarial embryos were badly infected, having numerous vesicles in their parenchyma and extensive irregular protuberances on their surfaces; these contained large numbers of what appeared to be developing stages of spores. However, no fully developed spores were found in the larval stages of *D. flexicaudum* in any of these 5 experimentally infected snails.

We concluded from this experiment that the ingestion of the *Nosema* spores had produced infections in the strigeoids harbored by these snails. It seemed to us of special significance that in all the snails that had ingested the spores, the hyperparasites in the larval strigeoids were at about the same stage, with large numbers of spores well along in development. This suggested that further experimental infections lasting a few more days might show the complete development of the spores.

Experiment II

On July 9, 1957, we found a heavy infection of *Nosema* sp. with large numbers of spores in the larval stages of *Apatemon* sp. (*Cercaria burti*) in *Helisoma trivolvis*. At this time we had in the laboratory 18 adults of *S. emarginata angulata* with mature infections of *D. flexicaudum* from various locations on the shores of Douglas Lake. Nine of these snails were exposed for 2 days in small dishes to large numbers of the spores of *Nosema* sp.; the other 9 were kept as controls.

Eight of the controls and 5 of the experimentally infected snails lived until the date of examination 20 to 24 days later. In 2 of the control snails no evidence of microsporidian infection could be found; 5 of the other controls had very light infections with only a small proportion of the cercarial embryos showing the vesicle stage. The other one had a moderate infection with quite a number of cercarial embryos having both vesicles and developing spores. However, in this snail only a small proportion of the daughter sporocysts were hyperinfected and no mature spores were present.

Two of the snails that had been exposed to the *Nosema* spores were examined 20 and 21 days after the beginning of exposure. Their *D. flexicaudum* infections had heavy hyperinfections with *Nosema* sp. Only a few of the daughter sporocysts were not infected, and only a small number of normal cercariae were being produced. Enormous numbers of mature spores were present in the daughter sporocysts and cercarial embroys, which were very abnormal in appearance. In the other 3 experimentally infected snails which were examined 21 to 24 days after first exposure to the spores, all the daughter sporocysts seemed to be infected and almost no normal cercariae were present. These 3 snails had been isolated in bottles of water to check cercarial production just prior to examination; 1 gave off no cercariae and the other two, 4 each. The daughter sporocysts and cercarial embryos in all 5 of these snails contained very large numbers of mature spores, and were greatly modified in appearance.

This experiment not only proved conclusively that hyperinfection of larval stages of strigeoids with *Nosema* sp. can be accomplished by feeding the spores to the snails, but also showed that in heavy experimental infections development of this microsporidian with the production of large numbers of spores can be completed in 21 days.

Experiment III

Seven juveniles of *S. emarginata angulata* which had mature infections of *D. flexicaudum* were exposed on July 31, 1957, to large numbers of the *Nosema* spores from one of the experimentally infected snails of experiment II. Six of these snails lived and were examined at intervals from August 7th to 23d. This experiment was planned to show the time needed for the development of mature spores.

1 (7 days). In this snail a large proportion of the cercarial embryos contained varying numbers of the vesicles, which varied considerably in size. They could also be seen in the walls of some of the daughter sporocysts. A few of the sporocysts and a number of the cercarial embryos had the small rounded protuberances on their surfaces which contained developing spores (fig. 1).

1 (10 days). In this snail the presence of *Nosema* infection was doubtful. Two mother sporocysts were found containing a few opaque granular daughter sporocyst embryos which appeared to contain a few vesicles. There was no certain evidence of infection in the daughter sporocysts and cercarial embryos, although some appeared to have a few vesicles and granules.

1 (13 days). The stages of *D. flexicaudum* in this snail had a few abnormal daughter sporocysts, containing cercarial embryos with vesicles and surface protuberances with developing spores. Also, a few *Nosema* spores that appeared to be fully developed were present.

1 (16 days). About three-fourths of the daughter sporocysts in this snail and

about 50 to 60 percent of the cercarial embryos that were freed by teasing showed hyperinfection with *Nosema* sp. All stages (vesicles, surface protuberances, and developing and mature spores) were present in both the daughter sporocysts and cercarial embryos, many of which were very abnormal in appearance. Some of the cercarial embryos had only the vesicles while others had both vesicles and developing and mature spores. Spores were also present in some of the germinal masses.

1 (19 days). The mother sporocyst of *D. flexicaudum* in this snail had fully developed spores in projections on its surface, and daughter sporocyst embryos inside of the mother also showed infection. Immature daughter sporocysts that had migrated from the mother were also infected with all stages, including mature spores. Most of the mature daughter sporocysts were greatly enlarged and opaque and most of the cercarial embryos were badly infected, some with only vesicles and others with all stages including mature spores.

1 (23 days). A large proportion of daughter sporocysts and cercarial embryos in this snail were infected with the microsporidian. Some of the daughter sporocysts were almost empty, and some contained much unicellular material which appeared to come from broken down germinal masses and small cercarial embryos. Numerous mature spores were present in the daughter sporocysts and cercarial embryos. There were only a few normal well developed cercarial embryos and germinal masses.

This experiment suggested that the complete development of *Nosema* sp. with the production of mature spores might take place in 13 days in the strigeoids in snails exposed to massive infection with the spores. However, this could not be considered as definitely proved, since the snails came from places where natural infections with *Nosema* sp. were present. In such experimental infections it seems evident that so many spores might be ingested by the snail that most, if not all, of the daughter sporocysts would be invaded by the sporoplasms that escaped from them; also some of the daughter sporocysts might be infected before leaving the mother.

Experiment IV

On August 3, 1957, twenty-five *S. emarginata angulata* with mature infections of *D. flexicaudum* were exposed to spores of *Nosema* sp. from 4 of the experimentally infected snails of experiment II. The snails to be infected were placed in a battery jar 7 inches in diameter in water 1⅝ inches in depth. The *Nosema* spores were put on the bottom of the container and on lettuce that was given the snails to eat. The snails were kept in this container from 11 A.M. to 7 P.M. They were stirred up several times and were seen to feed actively on the lettuce and on the deposits on the bottom of the container. It was evident that they must have ingested large numbers of spores. Twenty-five other snails of the same species with mature infections of *D. flexicaudum* were kept as controls, being given no exposure to *Nosema* spores.

Sixteen of the control snails survived and were examined.

1 (12 days). About 5 percent of the cercarial embryos of *D. flexicaudum* in this snail were infected with *Nosema* sp. with vesicles and some surface protuberances.

10 (24 days). In 9 of these snails no infection was found in the larval trematodes, but in the other there was a heavy infection with all stages including spores

in the daughter sporocysts and cercarial embryos.

5 (26 days). These 5 snails had very light infections with *Nosema* sp. with a very few cercarial embryos which contained a few vesicles.

Fourteen of the snails that were exposed to the *Nosema* spores survived for examination.

2 (12 days). In 1 of these snails some of the daughter sporocysts had opaque areas; about 5 percent of cercarial embryos were hyperinfected with many vesicles which produced abnormal shapes. The 2nd of these 2 snails had a somewhat heavier infection, with one-fourth to one-third of the cercarial embryos abnormal. All stages of the microsporidian were present including large numbers of spores both in the daughter sporocysts and cercarial embryos.

2 (24 days). Both these snails had heavy infections in all stages with many spores.

8 (25 days). The trematode larvae in all these snails showed infections including all stages and many spores. One of these snails had precociously developed metacercariae of *D. flexicaudum* in part of its daughter sporocysts; some of these contained mature *Nosema* spores (fig. 5).

2 (26 days). The *D. flexicaudum* infections in both these snails showed *Nosema* infections with spores. One of them had also some precociously developed metacercariae with mature *Nosema* spores.

This experiment gives further evidence that hyperinfection with *Nosema* of the strigeoids is accomplished by the ingestion of the spores by the snails. In all except 1 of the control snails in this experiment little or no natural infection with *Nosema* was found, while all the experimentally infected snails acquired rather heavy infections. In 1 snail spores were present after 12 days. Two of these experimentally infected snails had precociously developed metacarcariae of *D. flexicaudum*, some of which contained *Nosema* infections with spores.

Experiment V

Toward the end of the summer of 1957 a number of specimens of *Lymnaea stagnalis* were exposed to the cercariae of *Cotylurus flabelliformis* so that they would have various stages of the metacercariae of this strigeoid on which to try experimental infection with *Nosema* sp. These snails, infected only with the metacercariae of *C. flabelliformis* were exposed in a battery jar to enormous numbers of *Nosema* spores on lettuce for 26 hours. They consumed the lettuce very rapidly and also fed on the scum on the bottom of the jar so that they must have ingested very large numbers of the spores. As in experiment IV, the spores came from 4 heavily infected snails of experiment II. Nine of these snails survived for examination.

3 (11 days). In these 3 snails there were a number of actively motile unencysted metacercariae of *C. flabelliformis* which had *Nosema* infection in the vesicle stage.

1 (15 days). The metacercariae, both unencysted and encysted, in this snail had all stages including vesicles, developing spores and a few mature spores.

1 (26 days). This snail contained both actively motile unencysted metacercariae which had many spores and encysted tetracotyles with all stages.

4 (28 days). In these 4 snails the metacercariae of *C. flabelliformis* were infected with all stages.

The most interesting result of this experiment was the experimental infection with *Nosema* sp. of the young actively motile metacercariae of *C. flabelliformis*. While the encysted tetracotyles of *C. flabelliformis* in these snails might possibly have been present when the snails were brought into the laboratory, the young unencysted stages were undoubtedly experimentally introduced. This is proof that infections of *Nosema* sp. can produce mature spores in 15 days.

Encysted metacercariae (tetracotyles) of *C. flabelliformis* infected with *Nosema* sp., whether naturally or experimentally acquired, characteristically appear very dark and opaque. They usually show a folding and wrinkling of the cyst wall (figs. 4 and 6). Early stages of the infection, particularly in the developing metacercariae, may appear as rounded masses of equal sized small refractile bodies, which are attached to the outer tissue layers and project into the cavities. These masses are similar to those sometimes seen attached to the inner walls of the strigeoid sporocysts. We have considered them to be a stage of schizogony.

Experiment VI

In this experiment, 30 *S. emarginata canadensis* with mature infections of *D. flexicaudum* collected on July 18, 1958, on the shore of Lake Huron near Cheboygan, Michigan, were used to test the longevity of the spores of *Nosema* sp. Part of the spores used were collected from both natural and experimental infections during the summer of 1957. They had been pooled, put into water and kept under refrigeration except for two 4-day periods of transportation between Michigan and New York City.

On July 21, 1958, 10 snails of the above group were exposed to these 1957 pooled spores on lettuce in an aquarium for 2 days. Ten other snails of the group were exposed in the same way to spores collected earlier in the summer of 1958 and 10 were kept as controls.

These snails were kept in three different aquaria. On August 11, 1958, these aquaria were examined for the presence of free cercariae. The aquarium in which the controls were kept and that containing the snails exposed to the 1957 spores of *Nosema* had many cercariae swimming around in the water. The aquarium containing the snails exposed to the spores collected in 1958 had only a very few cercariae.

Nine of the control snails survived for 21 days, when they were examined. Two of them showed no evidence of *Nosema* infection; 6 had only a very small percentage of the cercarial embryos with the vesicle stage. In the other control snail moderate infection was present which had almost reached the spore stage.

Eight of the snails exposed to the 1957 spores survived for examination.

3 (21 days). In 2 of these snails the strigeoids had mature *Nosema* infections with spores, while in the other there were only a few abnormal embryos and no spores were seen.

1 (22 days). A few of the sporocysts of *D. flexicaudum* in this snail were badly infected, showing both the early vesicle stages and mature spores (figs. 2, 3), but the majority of the daughter sporocysts and cercarial embryos appeared normal.

4 (31 days). Infections with mature spores were present in the strigeoid infections in all 4 of these snails.

Eight of the snails exposed to the spores of *Nosema* collected in 1958 survived for examination.

4 (22 days). In 2 of these 4 snails the majority of the daughter sporocysts of *D. flexicaudum* were heavily infected with all stages of *Nosema* sp., while in the other 2 snails there were a smaller number of daughter sporocysts with very heavy infections with mature spores.

4 (31 days). In 3 of these snails all the strigeoid sporocysts were infected with *Nosema* in the spore stage. In the other snail there were many normal cercarial embryos present, although almost all the sporocysts appeared to be at least partly infected with *Nosema* in the spore stage.

The above experiment demonstrates that spores of *Nosema* sp. can remain viable for at least a year in water at refrigerator temperatures. Although the experimental infections produced with the 1957 spores were not nearly as heavy as those produced from the spores collected in 1958, it is evident that some of the spores had remained infective over the winter; 7 of the 8 snails exposed to 1957 spores had infections with mature spores, while mature spores were found in none of the control snails.

Experiment VII

At different times during the summer of 1958 snails with strigeoid infections were exposed to large numbers of spores of *Nosema* sp. collected from both natural and experimental infections early in the summer.

A. Nine *S. emarginata angulata* with mature infections of *D. flexicaudum* were exposed to large numbers of *Nosema* spores.

After 21 days 7 of these snails were isolated in bottles to check the escape of cercariae. In 4 of them the escape of the cercariae was greatly reduced, which gave evidence that a large proportion of the cercarial embryos were affected. The 9 snails were examined at intervals of 21 to 42 days after exposure to the spores. Their infections with *Nosema* sp. varied considerably in intensity but large numbers of spores were present in all of them. In the snail examined 42 days after exposure a large proportion of the cercarial embryos were almost completely destroyed by the *Nosema* infection. In the badly infected sporocysts in this snail there were almost no stages of *Nosema* in the sporocyst wall, but the cercarial embryos contained enormous numbers of spores. There were also present some precociously developing metacercariae which still had tails attached. These were heavily infected with the stages of *Nosema* including large numbers of spores (fig. 5).

B. An adult of *S. emarginata angulata* with a mature infection of *Cercaria laruei* was examined 21 days after exposure to *Nosema* spores. The majority of the daughter sporocysts were heavily infected with the stages of *Nosema* including large numbers of spores. There were also present in this snail some encysted metacercariae of *Cotylurus flabelliformis* which showed the characteristic appearance of *Nosema* infection (opacity, wrinkling of the cyst wall, and the presence of mature spores (figs. 4 and 6)).

C. An adult *Physa* sp. with a mature infection of *P. minimum* (*C. physae*) was examined 21 days after exposure to *Nosema* spores. Its larval stages had only a moderate infection. The infection was unusual in that most of the mature spores were in the sporocyst walls and that the cercarial embryos, although having many of the earlier stages, contained but few spores.

D. An adult of *S. emarginata angulata* with a mature infection of *C. emarginatae* was examined 22 days after exposure to the *Nosema* spores. The daughter sporocysts and the contained cercarial embryos were heavily infected with all stages in-

cluding large numbers of mature spores.

This experiment demonstrates experimental infection with *Nosema* in 4 different species of strigeoids. The infections varied considerably in intensity but in all of them large numbers of mature spores were produced. It also gives some information on the way in which the larval trematode infection in the snails can be almost completely destroyed by the *Nosema* hyperinfection.

DISCUSSION

The 7 experiments presented above prove conclusively that hyperinfection with the microsporidian, *Nosema* sp., can be transmitted to the strigeoids harbored by snails by feeding the spores to the hosts. Experimental infections that ran for as long as 15 days always produced spores. The finding of spores in some of the experiments from 11 to 13 days after infection may have possibly been due to the presence of previous natural infections in the snails. In addition to mother and daughter sporocysts and cercarial embryos the metacercariae (diplostomula) of *D. flexicaudum*, which developed precociously (cf. Olivier, 1940) and the metacercariae (tetracotyles) of *C. flabelliformis* were experimentally infected with all stages of the microsporidian (figs. 4 and 6).

Our studies on experimentally produced infections with *Nosema* sp. have so far given us no information on the escape of the sporoplasms from the spores ingested by the snails or how these stages reach and penetrate into the larval strigeoids; nor do we have any evidence that *Nosema* infection passes from sporocyst to sporocyst in the snail's digestive gland. The earliest stages of the life cycle of *Nosema* sp. we have seen are small vesicles containing minute granules, which are most clearly seen in the parenchyma of small cercarial embryos. The later stages of sporogony show up best in protuberances on the surface of sporocysts, cercarial embryos, and metacercariae. We have never found any stages of this microsporidian in the tissues of snails.

The very high percentage of the strigeoid infections in the snails of the Douglas Lake region that are hyperparasitized by *Nosema* sp. is very surprising. More surprising still is the fact that in all but a very small number of these infections only a small proportion of the sporocysts are infected, and mature spores are not produced. In fact, in most of the snails examined both in the early and late summer the few sporocysts and cercarial embryos that were infected with *Nosema* sp. contained only the vesicles with granules, which we consider to be early stages of schizogony. In the experimentally produced infections, on the other hand, a large proportion of the daughter sporocysts and the cercarial embryos in them were quickly infected, mature spores developed in 15 days and probably slightly earlier, and by 21 days were present in enormous numbers. One possible explanation of this type of development in experimental infections is that the snails ingest such enormous numbers of spores that rapid infection is produced in most of the strigeoid sporocysts, either while they are still in the mother or after they have migrated to the digestive gland of the snail. Then the number of sporoplasms that invade the sporocysts and start to develop is so great that they prevent the development of any resistance, which lets the life cycle be carried through very quickly with the development of large numbers of spores. On the other hand, it seems necessary to postulate in most natural infections the ingestion by the snails of small numbers of spores

which would permit the development of a resistance of the larval trematodes against infection with the microsporidian. This might prevent the development in most of the daughter sporocysts and hold back the completion of the life cycle with the formation of mature spores.

SUMMARY

It has been demonstrated experimentally that *Nosema* sp., a hyperparasite in strigeoid trematodes, can be transmitted by feeding its spores to snails. Very large numbers of spores were used and almost all of the experimental infections were heavy, affecting most of the strigeoid larval stages. The development was rapid and spores began to be produced by about 2 weeks after exposure; by 3 weeks spores were present in enormous numbers. No evidence has been obtained either from experimental or natural infections that *Nosema* sp. ever develops in the tissues of snails. In addition to mother and daughter sporocysts the infection was also transmitted to precociously developed metacercariae (diplostomula) of *Diplostomum flexicaudum* and to both immature and encysted metacercariae (tetracotyles) of *Cotylurus flabelliformis*. *Nosema* spores that had been kept over the winter in water at refrigerator temperatures were still able to produce experimental infections. The rapid and complete development of the experimental hyperinfections with *Nosema* sp. is in striking contrast to the type of development in natural infections, the great majority of which have only early stages in a small proportion of the larval trematodes. A tentative hypothesis suggested to explain this difference is that some type of resistance is developed in the natural infections, most of which are probably produced by the ingestion of only a few spores; in contrast, with the enormous numbers of spores ingested by the snails in the experimental infections the resistance may have been prevented from developing.

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Explanation of Plate I

Figure 1. Cercarial embryo of *D. flexicaudum* from experiment III infected with *Nosema* sp. Shows developing spores in protuberances on its surface.

Figure 2. Cercarial embryo of *D. flexicaudum* infected with *Nosema*, showing stages of sporogony on its surface.

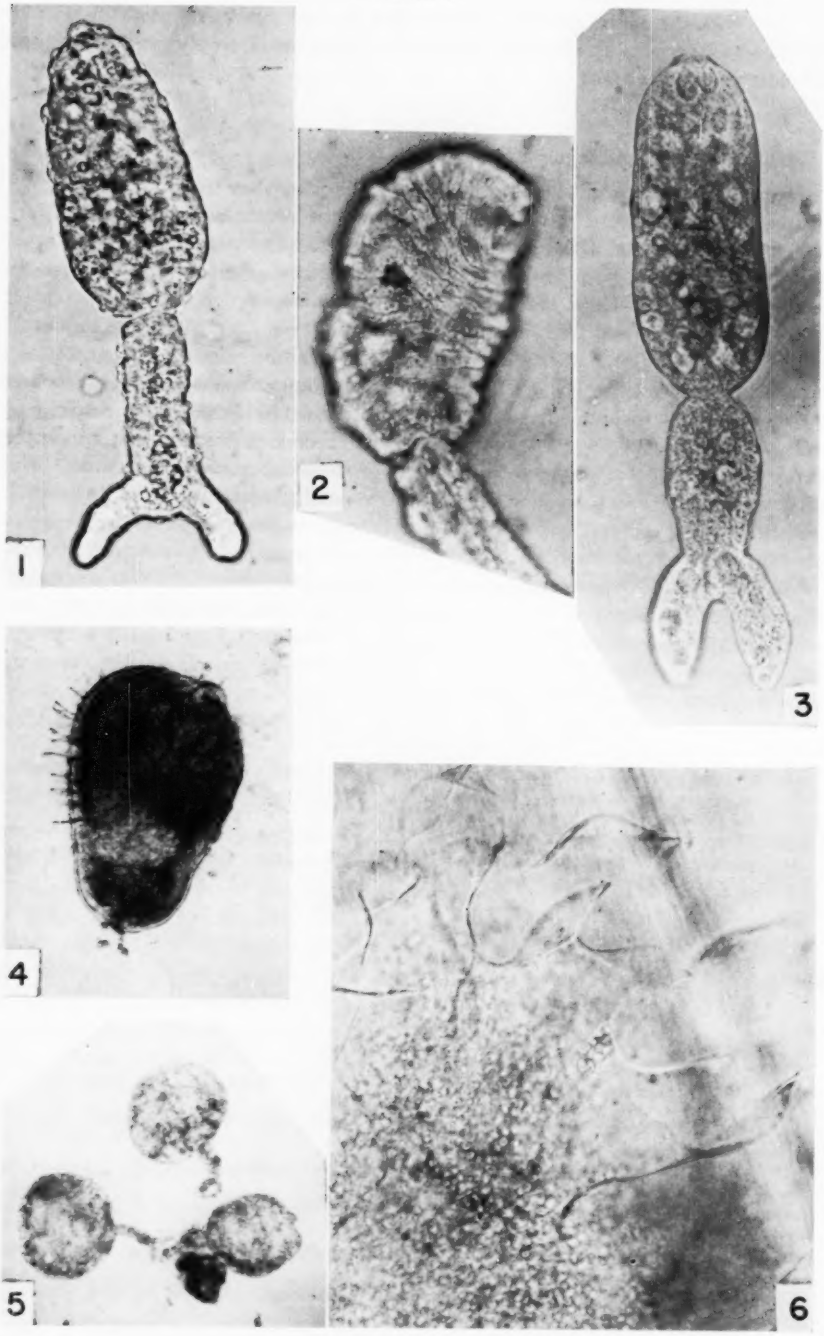
Figure 3. Cercarial embryo of *D. flexicaudum* with numerous vacuoles produced by *Nosema* infection in its body and tail.

Figure 4. Encysted tetracotyle of *C. flabelliformis*, showing wrinkling of cyst wall produced by *Nosema* infection.

Figure 5. Precociously developing metacercariae of *D. flexicaudum* infected with *Nosema* sp.

Figure 6. High power surface view of figure 4 showing folding of the cyst wall and mature spores.

PLATE I



THE LIFE CYCLE OF *PLAGIORCHIS VESPERTILIONIS PARORCHIS*, N. SSP., (TREMATODA: PLAGIORCHIIDAE), AND OBSERVATIONS ON THE EFFECTS OF LIGHT ON THE EMERGENCE OF THE CERCARIA

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Many *Lymnaea stagnalis* L. collected from Cascade Lake, Orcas Island, Washington, were found to shed large numbers of a species of xiphidiocercaria containing refractile granules. They resembled the cercariae of *Plagiorchis* as described by McMullen, 1937, and subsequent experiments proved that they were the larval stages of what appears to be an undescribed subspecies of *Plagiorchis vespertilionis* (Müller, 1784) Braun, 1900.

Encystment was induced experimentally in trichopterous larvae, dragonfly nymphs, ephemerid larvae, and *Culex* mosquito larvae. All were collected in the vicinity of Portland, Oregon, where *Lymnaea stagnalis* is not known to occur, and a series of such insects were dissected to demonstrate that all were free of trematode cysts of this type. Adult flukes were obtained by feeding infected insects to white mice, and proven to be a common species found in small *Myotis* bats on Orcas Island.

This work was supported by Contract Nonr 1805(00), NR 130-342, between the Office of Naval Research, Department of the Navy and Portland State College. The writer also wishes to acknowledge the assistance of Donald J. Moore for his help with certain aspects of the investigation. Further he is under special obligation to Dr. Franklin Sogandares-Bernal for furnishing a series of drawings of the position of organs in *Plagiorchis vespertilionis* from Korean bats. Dr. Murray L. Johnson of Tacoma, Washington, kindly identified the bats, specimens of which numbered 6922 to 6937 are now in the Puget Sound Museum of Natural History.

METHODS

Snail hosts transported in glass jars aerated with oxygen generators survived the 300-mile trip from Orcas Island to Portland in excellent condition. In the laboratory they were separated into shallow white-enameled pans covered with glass. The snails survived and could be reared from eggs in such pans if the bottoms were provided with fine gravel and water was maintained at a level not much higher than the diameter of the snails. Food was provided by maple leaves first placed for a minute in boiling water to soften them. Tap water which had been allowed to stand in large jars for at least 24 hours was quite satisfactory as a substitute for pond water. To check snails for infections, they were isolated overnight in shell vials 2 by 9 cm in size.

Cercariae were studied alive with and without neutral red stain. Those used for measurements first were lightly stained with neutral red and then killed in hot 10 percent formalin. Adult trematodes were fixed in Gilson's fluid after slight compression under a cover glass, and then stained with borax carmine and fast green or with Ehrlich's acid hematoxylin.

For the experiment on the effect of reversal of light and dark periods, light-tight cabinets were employed. These were furnished with fluorescent lights to minimize temperature changes and constructed for adequate ventilation. The lights were controlled by automatic time switches.

Measurements are in millimeters unless otherwise indicated. Figures 1 to 15 are of *P. vespertilionis* and were drawn with the aid of the camera lucida.

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Plagiorchis vespertilionis parorchis, n. ssp.
(Figs. 1–5)

Diagnosis: Body somewhat flattened, 1.55 to 1.80 long by 0.65 to 0.68 in maximum width, anterior half with small spines. Oral sucker subterminal, 0.160 to 0.186 long by 0.161 to 0.180 wide; acetabulum 0.144 to 0.174 in diameter, its anterior margin about 0.5 from anterior end of body. Suckers equal in size or acetabulum slightly smaller. Sucker ratio in specimens from mouse 1 : 1 to 1 : 108, from bats 1 : 1.09 to 1 : 1.30. Pharynx about 0.1 in diameter; prepharynx not evident. Esophagus very short, followed by well-developed ceca extending to near posterior tip of body; testes average 0.17 in diameter, nearly opposite but occasionally more oblique. Ovary rounded, 0.120 to 0.145 in diameter, on left side of body, usually just posterior to level of acetabulum. Acetabulum connecting with enlarged part of oviduct serving as a fertilization chamber. True seminal receptacle absent. Vitellaria in compact follicles of several cells each, mostly extracecal, extending from near posterior part of body to anterior margin of acetabulum or occasionally to a level midway between the suckers; never confluent in region of acetabulum, hence typical of the subgenus. Cirrus sac well developed, elongate, curved around margin of acetabulum or dorsal to it, and extending a short distance posterior to that sucker; containing seminal vesicle and long ejaculatory duct leading to submedian genital pore immediately anterior to acetabulum. Penis protrusible, long, narrow, apparently without spines such as described by Park (1936) for *P. noblei*. Ten uterine eggs from whole mounts of flukes reared in mice measured 0.035 to 0.038 by 0.018 to 0.020; ten eggs from natural infections in *Myotis* 0.033 to 0.037 by 0.017 to 0.019.

Hosts: (natural) *Myotis lucifugus alascensis* Miller; (experimental) *Mus musculus* L.
Site: small intestine.
Locality: Cascade Lake, Orcas Island, San Juan County, Washington.
Type specimens: Holotype No. 39045, Helm. Coll. U. S. National Museum, paratypes in author's collection.

In being subequal, the suckers of *P. vespertilionis parorchis* are unlike those of *P. micracanthos* Macy, 1931, the other North American member of the group found in bats. It was thought desirable to designate the population of *P. vespertilionis* from the state of Washington as a subspecies rather than a species because of the variation in the position of the testes. In Washington specimens these organs tend to be nearly opposite, noticeable even in living material, whereas in Asiatic and European populations they are oblique. The testes of a small number of *P. vesperitilionis parorchis* are oblique in varying degree (fig. 16). In a preliminary note by the writer (1956) the Washington population was treated as a separate species.

To express quantitatively the difference between Asiatic and North American material, the angles formed by the testicular and longitudinal body axes were determined from camera lucida drawings of 21 specimens of *P. vespertilionis parorchis* and 22 *P. vespertilionis* from Korea. Outline drawings of the Korean material were provided by Dr. Sogandares-Bernal. The results (fig. 16) clearly demonstrate that the testicular angle of *P. vespertilionis parorchis* is greater, i. e., the testes are more nearly opposite than in the Korean material. A further difference is the small size of *P. vespertilionis parorchis* in comparison with European and Asiatic specimens.

Caballero y C. (1942) reports 3 specimens of *P. vespertilionis* from the bat *Tadarida brasiliensis* Geoffroy in Mexico, but there is not sufficient data to compare material with the subspecies under consideration. *P. bulbulii* Mehra, 1937, *P. russii* Mehra, 1937, and *P. clelandi* Johnston, 1916, all show strong resemblance to *P. vespertilionis* but only experimental work will determine their true status.

Cercaria (Fig. 7)

Diagnosis: Xiphidiocercaria of Polyadena group of Sewell (1922). Body of 15 cercariae killed in hot 1 percent formalin averaging 0.22 long by 0.092 wide. Fine cuticular spines on

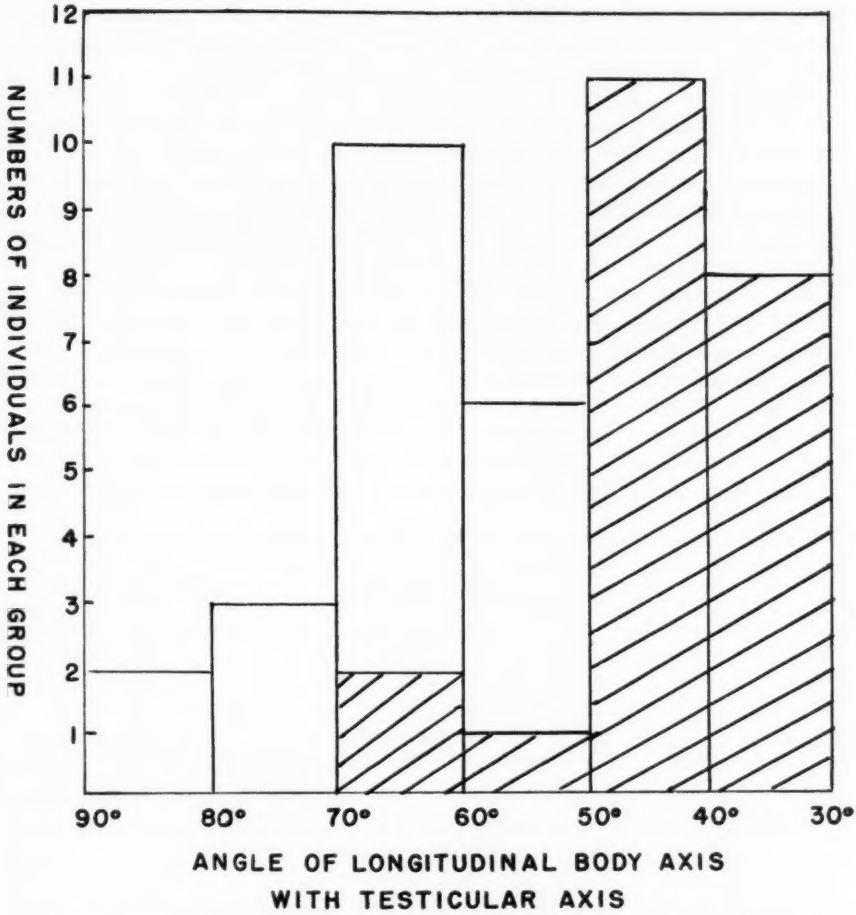

FIGURE 16. Diagram to show differences in testicular angle in two populations of *P. vespertilionis.* The clear columns at left represent frequencies for *P. vespertilionis parorchis;* those on the right, *P. vespertilionis vespertilionis* from Korea.

anterior third of body, no hair-like extensions of type described by Rees (1952) for *P. megalorchis.* Oral sucker 0.048 in diameter, stylet 0.030 long (fig. 14), ventral sucker averaged 0.031 in diameter. Round, refractile granules, characteristic of this group of cercariae, numerous, maximum diameters of 0.006 to 0.012, apparently larger than those seen by McMullen (1937) in the cercaria of *P. micracanthos,* less clumping than in that species, filling most of body except region anterior to mid-distance from oral sucker to ventral sucker. Pharynx approximately 0.015 in diameter, remainder of digestive tract obscured by granules and glands which also prevented observation of most of excretory system. Penetration glands largely obscured by granules and numerous smaller glandular cells filling most of posterior half of body. The tail much contracted and creased in living cercaria under a cover glass, measuring 0.15 long by 0.028 wide; 0.122 to 0.174 long by 0.020 to 0.035 wide in formalin-killed specimens.

Host: *Lymnaea stagnalis* L.

Locality: Cascade Lake, Orcas Island, Washington.

The cercaria of *P. vespertilionis parorchis* seems to respond very little to

changes in light intensity. It often swims near the bottom of the container and frequently creeps for brief periods. At room temperature it may live as long as 36 hours and be able to penetrate mosquito larvae longer than 26 hours.

Normally cercariae emerge almost entirely at night. To obtain more information about the time of shedding, 5 naturally infected snails were isolated in fingerbowls and kept in a dark room during the night. At hourly intervals each snail was transferred to a fresh bowl of water and the cercariae that had emerged were killed after being lightly stained with neutral red, and counted. All transfers were carried out in darkness. It was observed (fig. 17) that the 5 snails shed their maximum numbers of cercariae during the hours preceding 9 p.m., 10 p.m., 10 p.m., 11 p.m., and 1 a.m., respectively, and these numbers were 280, 1121, 1998, 786, and 585.

To determine the effect of reversing the normal light and dark cycle, another group of eight infected snails were isolated in individual finger bowls, placed in a light-tight cabinet, and subjected to 12 hours of light at night (6 p.m. to 6 a.m.) and the same period of darkness during the day. The snails were transferred to fresh dishes at 9 a.m. and 4:30 p.m. The experiment was run 2 days before cercarial counts were made. As shown in table I, the snails kept in the dark during the day shed more than 3 times as many cercariae as did those exposed to light at night,

TABLE I. *Numbers of cercariae shed by snails after artificial night and artificial day.*

Snail	6 PM Collection (after artificial night)	6 AM Collection (after artificial day)
1	Less than 50	Snail dead. Few cercariae
2	256	125
3	353	48
4	450	148
5	648	174
6	176	76
7	127	78
8	124	39
Totals	2135	688

whereas previous to the experiment they had shed practically no cercariae during the day. Temperature of the water during the dark period was slightly higher than during exposure to artificial light, whereas under normal conditions cercariae were shed almost entirely at night when temperature is lower. Light thus seems to be the principal factor in determining the time at which this cercaria is shed. When shed in large numbers the cercariae tended to aggregate in clumps of 100 or more.

By the end of June a few infected snails may be found, and the incidence increases by the end of the summer. A series of 176 snails collected at Cascade Lake on August 4 and 5, 1956, showed an infection rate of about 8 percent. Incidence of this infection in snails has been noted since 1950 when observation at Cascade Lake first began. In 1954, 12.4 percent of 370 *Lymnaea stagnalis* from the lake were infected; in 1955, 14.3 percent of 294.

Sporocyst (Figs. 10–13)

The cercariae are produced in elongated, yellowish-orange sporocysts about 0.7 by 0.11. No case of precocious development of metacercariae in sporocysts could be found and no adults were obtained by feeding quantities of sporocysts to mice and hamsters. In this respect, the cercaria of *P. v. parorchis* is like that of *P. micracanthos*, but differs from *P. muris* Tanabe, 1922, as reported by McMullen (1937).

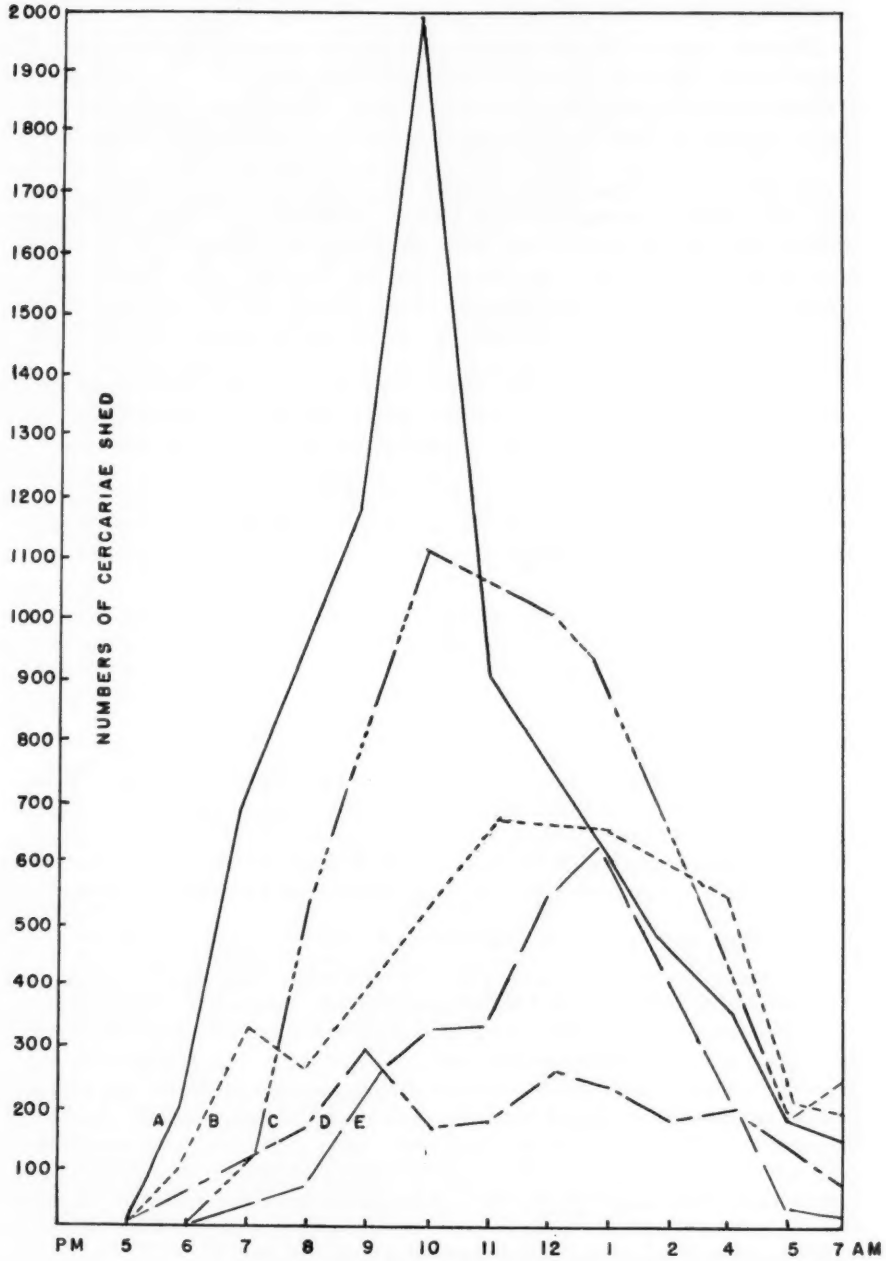

FIGURE 17. Shedding period of cercariae of *P. vespertilionis parorchis*. Letters represent numbers of cercariae from individual snails.

Experimental Infection of Insects

Mosquito larvae, caddis-fly larvae, and dragonfly nymphs were readily penetrated by these cercariae and encystment ensued shortly thereafter. To study penetration, small mosquito larvae were placed in water containing cercariae on a slide and were restrained by light cover-glass pressure. In addition to direct observation, motion pictures of the penetration process were made for later analysis.

The cercariae exhibited little if any attraction to the insect host, and often swam away after making temporary contact with it. However, those which on contact crawled leech-like over its surface for a time frequently remained to penetrate, stopping from time to time to test that surface with the stylet. After several minutes, the larva began the penetration process by forming with the stylet, aided no doubt by enzymes from the cephalic glands, a small opening through which the body slowly squeezed.

The cercaria often explored the surface of the siphon but entered its distal end. Once inside the insect, the tailless cercariae moved about in the hemocoel, disturbing various structures and frequently stimulated the insect to react violently. The time required for penetration was from 10 to 15 minutes, and encystment generally occurred 30 to 45 minutes thereafter with the formation of a thin cyst wall within which the body of a cercaria remained active for a time. Cysts formed in mosquito larvae remained viable in the pupae even when the latter were kept for an extended period at 4° C.

As small caddis-fly larvae frequently were killed by heavy exposure to many cercariae, care had to be exercised in using them for infection experiments. Although dragonfly nymphs were more resistant, they also were killed by massive exposure to cercariae.

Metacercaria (Figs. 6, 8, 9)

Newly formed cysts averaged 0.126 by 0.170 in size. After 6 days there was no appreciable change in size, but a second much thicker, irregular layer of host origin often formed in caddis-fly larvae and dragonfly nymphs but not in mosquito larvae (fig. 8). The anterior end of the metacercaria is finely spinose. The stylet was often retained for several days, but sometimes disappeared soon after encystment.

Experimental Infection of the Definitive Host

Ten white mice were fed insects experimentally infected with week-old cysts of *P. vespertilionis parorchis*. Of 2 fed infected dragonfly nymph tissue and examined seven days later, 1 was negative and the other contained 5 flukes with well-developed sex organs and sperm in the seminal vesicle, but no eggs. Eggs appeared in the feces of 2 others 10 days after they were fed infected caddis-fly larvae, but 6 days later no trematodes were found when the mice were killed and examined. Another mouse fed cysts from caddis-fly larvae yielded 7 large, mature flukes 16 days later. The worms were as large as any found in bats, the natural hosts. Three other mice fed such cysts were negative. Finally, 1 of 2 mice fed infected mayfly material produced 5 mature flukes after 15 days. The remaining mouse was negative, as were also 3 hamsters fed caddis-fly larvae containing week-old cysts.

SUMMARY AND CONCLUSIONS

A new subspecies of the bat fluke *Plagiorchis vespertilionis* and its life history

are described. The testicular angle is used to indicate subspecific differences in the position of the testes.

The cercaria, of the Polyadena group of xiphidiocercaria, develops inside sporocysts in *Lymnaea stagnalis* and encysts in mosquito larvae, caddis-fly larvae, mayfly larvae and dragonfly nymphs. Cercariae are shed almost entirely at night, but in a preliminary experiment it was found that shedding time could be largely reversed by changing periods of illumination.

Granules filling much of the body of the cercaria were judged to be somewhat larger than those of the cercaria of *P. micracanthos.*

Seven-day old metacercariae in caddis-fly larvae, mayfly larvae, and dragonfly nymphs proved to be infective when fed to white mice. Three hamsters and several white mice were refractory to infection. After 7 days in mice the worms had well-formed organs and sperm, but eggs had not yet appeared; in 16 days maximum size of mature worms was attained after which they soon were lost from the host.

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Explanation of Plates

Plate I

Figure 1. Type specimen, reared in mouse; dorsal view.

Figures 2–5. Mature specimens, to show variation in position of testes and distribution of vitellaria, drawn to smaller scale.

Figure 5. Testicular angle formed by testicular axis and longitudinal body axis.

Figure 6. Excysted metacercaria, lateral view.

Plate II

Figure 7. Cercaria, living specimen.

Figure 8. Encysted metaceacaria, living, surrounded by host reaction tissue. From caddis larva.

Figure 9. Excretory system as seen in live metacercaria.

Figures 10, 11, 12. Sporocysts drawn in outline.

Figure 13. Sporocyst showing cercariae.

Figure 14. Stylet of cercaria.

Figure 15. Portion of adult to show extruded cirrus.

PLATE I

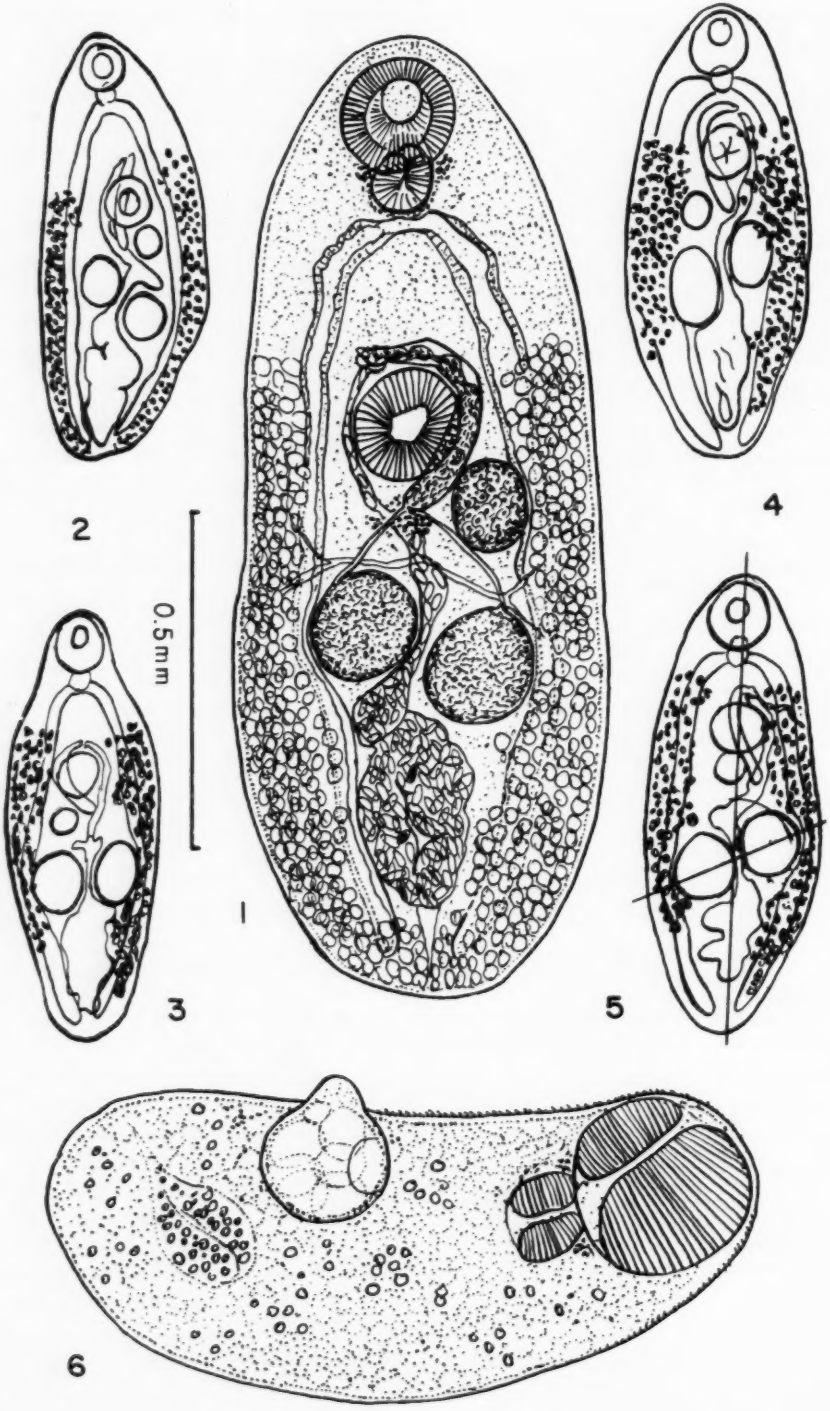
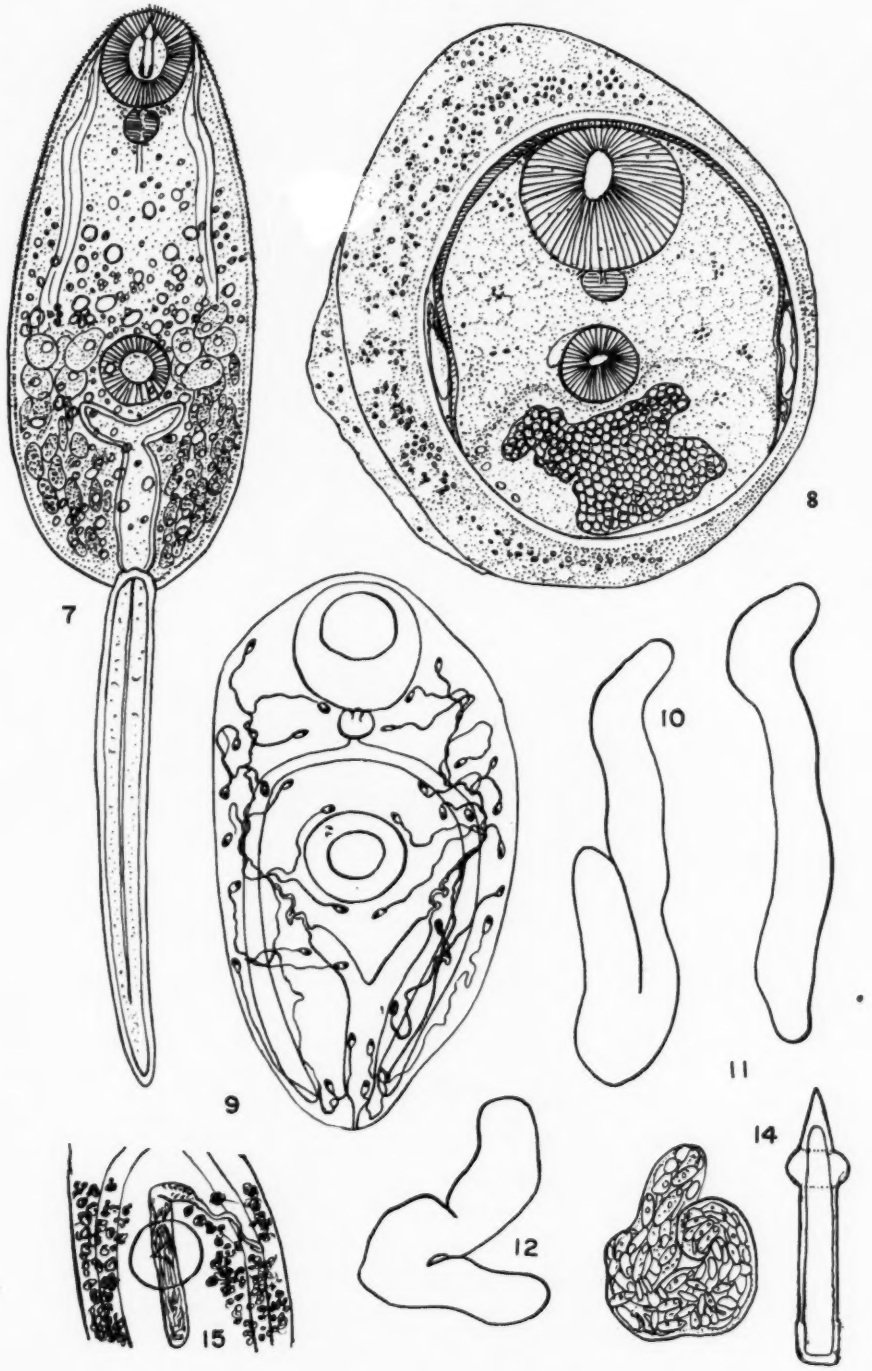


PLATE II



NEW RECORDS AND DESCRIPTIONS OF CHIGGERS (ACARINA: TROMBICULIDAE) ON BATS IN ALABAMA

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In assembling data for his doctoral dissertation at the University of Alabama, the junior author made a 7-year study of acarines of bats in Alabama. This report is based almost entirely on material which he collected in the course of that study.

Perissopalla, n. g.

Trombiculid larvae of subfamily Trombiculinae. Expanded, elongate sensillae with flagelliform setules. Palpal tarsus with 5 branched setae, a subterminala and a tarsala.

Type species: Perissopalla flagellisetula, n. sp.

The new genus differs from *Euschöngastia* and *Trombigastia* in the palpal tarsal setation (7 branched setae and a tarsala in each) and in the peculiar sensillae with flagelliform setules.

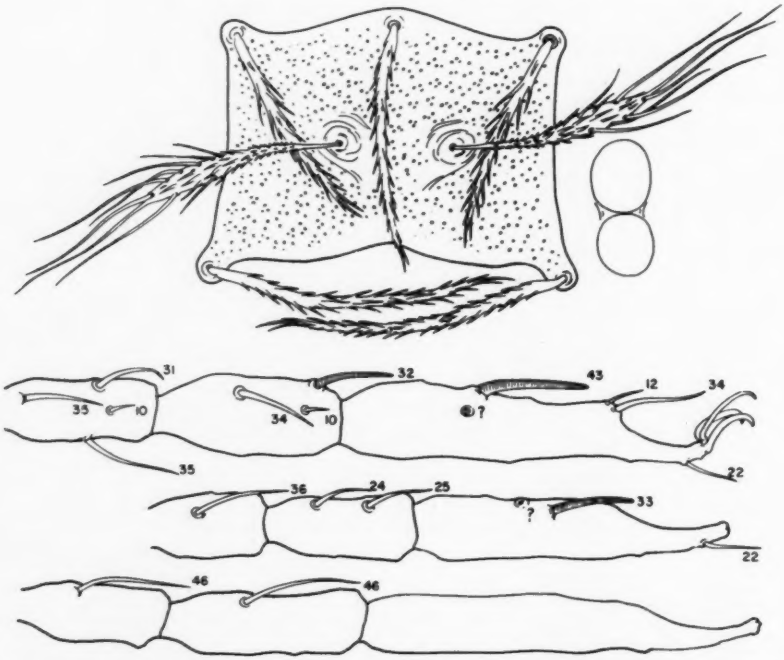

FIGURE 1. *Perissopalla flagellisetula*, n.sp. Scutum and specialized setae of legs with measurements in microns.

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Perissopalla flagellisetula, n. sp. (fig. 1)

Type data: Holotype and 9 paratypes, RML No. 34951, off bat, *Pipistrellus s. subflavus*, Cat Cave, Clarke County, Alabama, 25 August 1953; 1 paratype, RML No. 35034, some host, Six Mile Cave, Bibb County, Alabama, 8 January 1953.

Holotype and some paratypes in the Rocky Mountain Laboratory; other paratypes in the United States National Museum, the British Musem (Natural History), and the Chicago Natural History Museum.

Body: Ovate. Length and width of holotype, relatively unengorged, 464 by 278 microns. Eyes large, 2/2, nearly contiguous but not in a plate, subequal. Anus at 5th row of ventral setae.

Gnathosoma: Cheliceral bases, femoral and genual plates, capitular sternum punctate. Blades with tricuspid cap, no other dentition. Palpal setae large, B/B/NBB, the dorsotibial rarely forked; claw long, some specimens with 5, others with 4 prongs; tarsus with 5 heavily branched setae, a subterminala and a long tarsala. Galeal seta large, nude.

Scutum: Roughly rectangular with sinuous anterior and posterior margins, the latter deeply emarginate. Puncta small and numerous. Sensillae centered, long fusiform, with several extremely long flagelliform setules in addition to those of the usual type, the stems with short barbs distally. Setae of usual form. Scutal measurements of holotype: AW-83, PW-96, SB-31, ASB-42, PSB, 31, AP-66, AM-69, AL-60, PL-93, S-60.

Legs: Punctate, very long. Length of leg I, 505 microns; leg II, 425; leg III, 515. *Specialized setae*, very large: Leg I—3 genualae, microgenuala; 2 tibialae, microtibiala; tarsala, microtarsala proximal to it, sub- and parasubterminala, pretarsala. Leg II—genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, basifemur 2, telofemur 4, genu 3, tibia 6, tarsus 14. Leg III—coxa 1, trochanter 1, basifemur 2, telofemur 3, genu 3, tibia 6, tarsus 12. All tarsi with paired claws and long empodium.

Body setae: Dorsal setae 50 to 83 microns long, decreasing in length posteriorly, 2 humerals plus about 60, in irregular rows starting with 6 in the first. Ventral setae 2-2 sternals plus about 60, postanals like the dorsals.

Euschöngastia staffordi, n. sp. (fig. 2)

Type data: Holotype and 3 paratypes, RML, No. 35030, off bat, *Piapistrellus s. subflavus*, Six Mile Cave, Bibb County, Alabama, 8 January 1953; 1 paratype, RML No. 34967, same data; 5 paratypes, RML Nos. 34968 and 35032, same host and locality, 1 November 1952; 2 paratypes, RML Nos. 35022 and 35024, same host, Crump Cave, Blount County, Alabama, 15 February 1953; 2 paratypes, RML No. 35019, same host, Pratts Ferry Cave, Bibb County, Alabama, 30 January 1953; 8 paratypes, RML No. 35017, off *Eptesicus f. fuscus*, Goat Cave, Madison County, Alabama, 2 February 1953.

Holotype and some paratypes in the Rocky Mountain Laboratory; other paratypes in the United States National Museum, the British Museum (Natural History), the Chicago Natural History Museum, and the South Australian Museum.

Diagnosis: The shape and size of scutum, ovate sensillae, and AL > PL distinguish *Euschöngastia staffordi* from other North American species lacking a tibiala III and having 2 genualae I, a genuala III, subterminala, and trifurcate palpal tibial claw.

Body: Long-ovate. Length and width of holotype, partially engorged, 710 by 360 microns. Eyes 2/2, large, no plate. Anus at 4th row of ventral setae.

Gnathosoma: Usual structures punctate. Cheliceral blades small, with tricuspid cap. Palpal setal formula B/B/BNB; claw trifurcate; tarsus with 7 branched setae and a tarsala. Galeal seta branched.

Scutum: Rectangular with mildly sinuous margins, wider than long, punctate. Sensillae broad-ovate with conspicuous setules, bases anterior to PL's. AM posterior to AL's. Setae long and slender. Scutal measurements of holotype: AW—71, PW—101, SB—30, ASB—32, PSB—15, AP—39, AM—48, AL—87, PL—83, S—36.

Legs: Punctate. *Specialized setae:* Leg I—2 genualae, microgenuala; 2 tibialae (the distal blunt, the proximal acuminate), microtibiala; tarsala, microtarsala, sub- and parasubterminala, pretarsala. Leg II—genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—genuala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 7, tarsus 16. Leg II—coxa 1, trochanter 1, basifemur 2, telofemur 4, genu 3, tibia 6, tarsus 14. Leg III—coxa 1, trochanter 1, basifemur 2, telofemur 3, genu 3, tibia 6, tarsus 12.

Body setae: Dorsal setae 46 to 66 microns long, decreasing in length posteriorly, 2–10 plus about 28 in irregular rows. Ventral setae 2–2 sternals plus about 36, postanals similar to dorsals.

Anomalies: Several specimens have 2 anteromedian scutal setae.

Named for Dr. E. W. Stafford, Professor of Zoology and Entomology, Mississippi State University, retired.

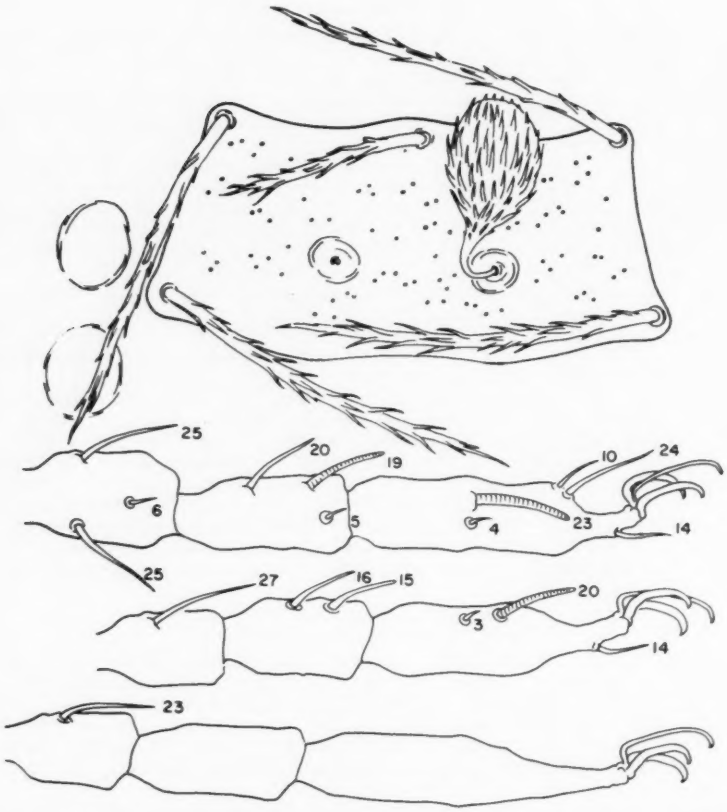

FIGURE 2. *Euschöngastia staffordi*, n.sp. Scutum and specialized setae of legs with measurements in microns.

Trombicula tibbettsi, n. sp. (Fig. 3.)

Type data: Holotype and 2 paratypes, RML No. 34266, off bat, *Mormoops megalophylla senicula*, Frio Cave, Uvalde County, Texas, 17 November 1956, Ted Tibbetts, collector. One paratype, RML No. 34960, off bat, *Myotis grisescens*, Collier Cave, Lauderdale County, Alabama, 27 September 1953; 1 paratype, RML No. 34962, *M. grisescens*, Sanders Cave, Conecuh County, Alabama, 9 August 1953; Jesse S. White. Eight paratypes, RML Nos. 34019 and 34021, off 2 bats, *Chilonycteris r. rubiginosa*, Tamana Hill Cave, Trinidad, B.W.I., 12 June 1956; 5 paratypes, RML No. 34665, off bat, *Mormoops tumidiceps*, same location, 20 November 1957; collected by personnel of Trinidad Regional Virus Laboratory, Port-of-Spain, Trinidad.

Diagnosis: The scutal shape (indented lateral margins, acute posterior angle), sensillae with 2 branches, lack of eyes, and angulate condition of palpi distinguish *T. tibbettsi* from other species of the group.

Body: Ovate. Length and width of holotype, engorged, 475 by 330 microns. Eyes absent. Anus between 4th and 5th rows of ventral setae.

Gnathosoma: Usual structures punctate. Cheliceral blades with tricuspid cap. Bases with prominent outer lateral angles. Palpal femora and genua also with pronounced lateral angles. Palpal setal formula: B/B/NNB, the femoral and genual much thicker than the tibial setae. Palpal claw trifurcate; palpal tarsus large, with 6 branched setae and a short tarsala. Galeal seta coarse, with few large branches.

Scutum: Pentagonal; punctate; acute posterior angle and sharply incurved lateral margins. Sensillae centered, with 2 long branches distally. Posterolateral setae thicker than the others.

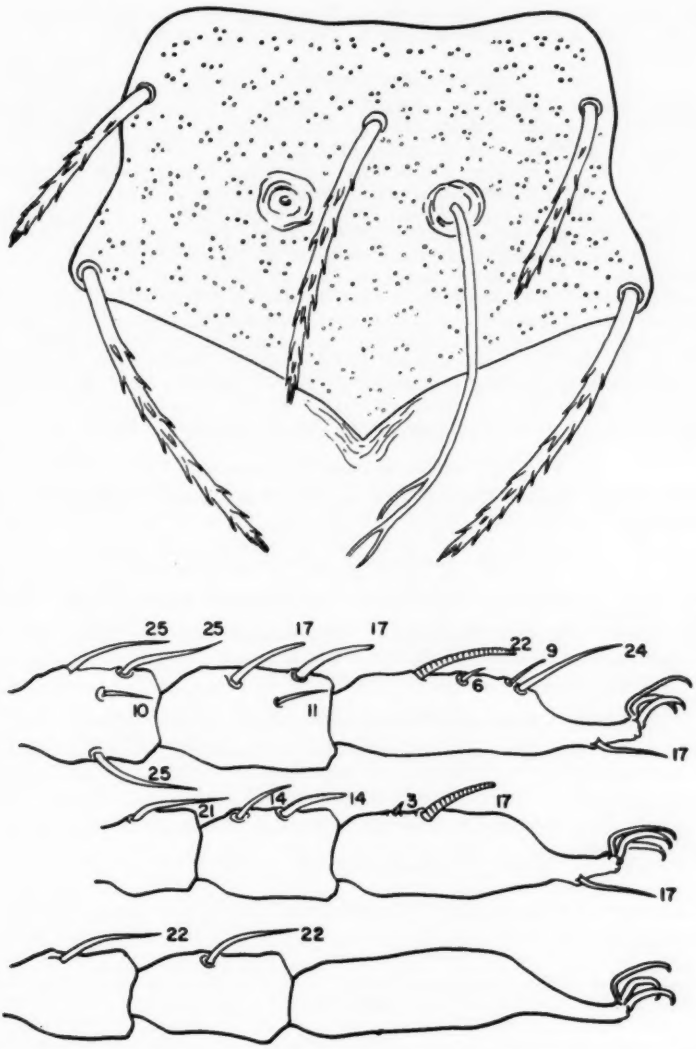

FIGURE 3. *Trombicula tibbettsi*, n.sp. Scutum and specialized setae of legs with measurements in microns.

AM posterior to AL's, all of these set considerably behind anterior margin. Scutal measurements of holotype: AW-62, PW-80, SB-22, ASB-30, PSB-28, AP-24, AM-41, AL-29, PL-45, S-. . .

Legs: Punctate. *Specialized setae:* Leg I—3 genualae, long microgenuala; 2 tibialae (proximal acuminate, distal blunt), long microtibiala; tarsala, microtarsala, sub- and parasubterminala, pretarsala. Leg II—genuala; 2 tibialae; tarsala, microtarsala, pretarsala. Leg III—genuala; tibiala. *Branched setae:* Leg I—coxa 1, trochanter 1, basifemur 1, telofemur 5, genu 4, tibia 8, tarsus 18. Leg II—coxa 1, trochanter 1, basifemur 2, telofemur 4, genu 3, tibia 6, tarsus 16. Leg III—coxa 1, trochanter 1, basifemur 2, telofemur 3, genu 3, tibia 6, tarsus 14.

Body setae: Dorsal setae 27 to 45 microns long, decreasing in length posteriorly; formula, 2-6-6-6-4-4. Ventral setae 2-2 sternals plus 32, postanals similar to dorsals.

Euschöngastia pipistrelli Brennan

Bibb County: From 6 *Pipistrellus s. subflavus,* Six Mile Cave, 5 October 1952 to 8 January 1953, 17 specimens identified; 1 *Myotis keenii septentrionalis,* same cave, 8 January 1953, 1; *P. s. subflavus,* Pratts Ferry Cave, 30 January 1953, 2.
Blount County: 1 *P. s. subflavus,* Bryant Cave, 1 November 1958, 1; same host, Crump Cave, 15 February 1953, 3; 3 same host, Dixson Cave, 22 February 1953, 11.
Calhoun County: 1 *P. s. subflavus,* Weaver Cave, 1 February 1953, 4.
Clarke County: 1 *P. s. subflavus,* Cat Cave, 25 August 1953, 24.
Jackson County: 1 *M. grisescens,* Racoon Creek Cave, 16 July 1954, 1.
Lawrence County: 1 *P. s. subflavus,* Salt Petre Cave, 27 July 1953, 2.
Madison County: 1 *P. s. subflavus,* Shelta Cave, 1 February 1953, 3.
Marshall County: 1 *P. s. subflavus,* Light Cave, 8 March 1953, 1.
Morgan County: 8 *P. s. subflavus,* Goat Bluff, 2 August 1953, 46; Walden Chapel, 2 August 1953, 1; Hughes Cave, 3 February and 26 July 1953, 19; Lemmon Cave, 8 March 1953, 2; McBride Cave, 25 July 1953, 16; Salt Petre Cave, 26 July 1953, 3; Woody Cave, 2 August 1953, 2.
St. Clair County: 3 *P. s. subflavus,* Greasy Cove Cave, 14 June and 14 July 1954, 17.

Euschöngastia hamiltoni Brennan

Tuscaloosa County: From 1 *Eptesicus f. fuscus,* attic, Tuscaloosa, 30 April 1959, 4 specimens identified.

Trombicula (Eutrombicula) alfreddugesi (Oudemans)

Conecuh County: From 1 *Myotis keenii septentrionalis,* Sanders Cave, 9 August 1953, 10 specimens identified.
Madison County: 1 *Eptesicus f. fuscus,* Goat Cave, 2 February 1953, 1.
Tuscaloosa County: 2 *Lasiurus borealis,* University, 12 August 1953, 19.
Choctaw County: 1 *L. borealis,* Emory, 24 August 1956, 2.

Trombicula (Eutrombicula) batatas (Linnaeus)

Lamar County: From 1 *Lasiurus borealis,* Vernon, 12 August 1956, 1 specimen identified.

Trombicula gurneyi Ewing

Bibb County: From 1 *Nycticeius humeralis,* Talladega National Forest, 15 August 1956, 1 specimen identified.
Geneva County: 1 *N. humeralis,* Geneva, 14 July 1956, 1.
Tuscaloosa County: 1 *N. humeralis,* 14 August 1956, 3.
Greene County: 1 *N. humeralis,* Union, 8 August 1956, 1.

Whartonia senase (Greenberg)

Tuscaloosa County: From 1 *Eptesicus f. fuscus,* attic, Tuscaloosa, 30 April 1959, 5 specimens identified.

SUMMARY

Described are *Perissopalla,* a new genus, and the new species *P. flagellisetula* off *Pipistrellus s. subflavus,* Alabama; *Trombicula tibbettsi,* holotype, off *Mormoops megalophylla senicula,* Uvalde County, Texas; other material off *Myotis,* Alabama, and *Mormoops* and *Chilonycteris,* Trinidad, B.W.I.; *Euschöngastia staffordi* off *P. s. subflavus* and *Eptesicus f. fuscus.* Alabama. Records are given for 6 other species.

NOTES ON THE MORPHOLOGY OF THE LARVA OF *PNEUMONYSSOIDES CANINUM* (CHANDLER AND RUHE, 1940) FAIN, 1955 (ACARINA: HALARACHNIDAE)

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Gamasid mites of the family Halarachnidae are parasitic in the respiratory tracts of mammals. Of the various genera in this family only *Pneumonyssoides* appears to be of importance in canine animals. *Pneumonyssoides caninum* has been reported as occurring in the nasal cavity and paranasal sinuses of dogs from various parts of the world. Although they live in the upper respiratory tract where they are not easily seen, no lesions or symptoms have been described that could be definitely attributed to their presence.

Fain (1955) described a new species, *Pneumonyssoides phacochoeri,* from the nasal cavity of the wart-hog and in this publication transferred *Pneumonyssus caninum* Chandler and Ruhe, 1940, to the genus *Pneumonyssoides.* He justified the inclusion of *P. caninum* in the new genus by his modification of the diagnosis of *Pneumonyssus* by Furman (1954). This revision was accepted by Strandtmann and Wharton (1958) and is included in their classification of mesostigmatid mites. These authors stated that the status of the 5 genera recognized in the family Halarachnidae Oudemans, 1906, was rather tenuous and was certainly not the final word. Furthermore, they indicated that very likely a separate genus will have to be erected for the mites for each of the various unrelated groups of hosts.

Chandler and Ruhe (1940) in their description of the female of this species, listed some of the morphological characteristics of the larva and included a description of the larval hypostome. Furman's account of the characteristics of the male was included in his revision of the genus. The entire larva of *P. caninum* is described here for the first time.

The following description of the larva is based on 10 specimens. All measurements are given in millimeters, and drawings were made from lacto-phenol-cleared specimens with the aid of a camera lucida.

General body shape: Oval; idiosoma length, 0.666 to 0.764 (average 0.725), width 0.485 to 0.533 (average 0.506). Gnathosoma roughly quadrangular, slightly longer than wide; closely associated with coxae I; tectum seldom distinct. Palpus (fig. 1, 2) with 4 apparent segments, the palpal tibia and palpal tarsus appear fused, and end with a single terminal spine; spinelets on all segments except coxae; number and position vary with segment. Chelicerae longer than palpi; not heavily chitinized. From dorsal aspect (fig. 2), terminal structure of chelicera with an articulated, blunt process positioned ventral to fixed process. A small spinous process below base of fixed digit. (Figure 3 indicates that these structures apparently carry through to the adult). Hypostome bifid, longitudinal division extending posteriorly to fold that demarcates thorax; corneous thickenings on anterior and outer edges, surface porose.

Dorsum (fig. 5): Dorsal plate absent; 10 pairs dorsal setae, 4 of these arranged in 2 longitudinal rows on the medial aspect of propodosomal region, graduated in increasing length posteriorly; 2 pairs lateral to 2 middle pairs of setae in series and medial to coxae II and III. Four pairs of setae located on opisthosoma. A median terminal filamentous postanal seta on

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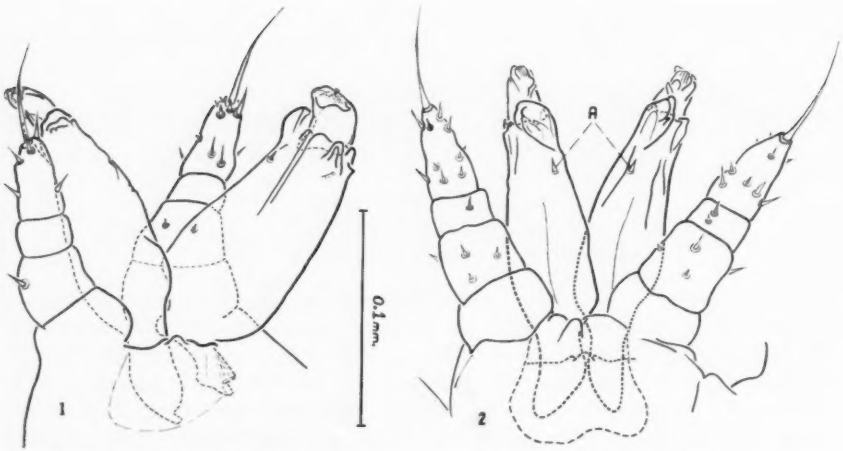

FIGURE 1. Chelicerae and palpi, larva, *P. caninum*, latero-ventral view. ×430.
FIGURE 2. Chelicerae and palpi, larva, *P. caninum*, dorsal view. ×430. Letter "A" indicates the spinous process near the base of the fixed digit of each chelicera that remains constant through the molt into the adult stage as seen in figure 3.

posterior margin of abdomen.

Venter (fig. 5): Anal and sternal plates absent. Three pairs of sternal setae, 1 pair of setae on opisthosoma, 1 pair of filamentous setae flanking median terminal filamentous postanal seta.

Legs: Resembling those of adult. Leg I closely associated with gnathosoma and longer than II and III. Tarsus I with pair of lightly sclerotized apposable claws articulating with terminal end of tarsus. Caruncle discernible. Tarsus I (fig. 4), posterior to claws, with pair of long sensory setae, each terminated by an arcuate hook. Setal arrangement of tarsus I similar to adult, including mid-dorsal oval area containing 3 very short triangular spinules. Pretarsus of legs II and III similar to adult.

Internal organs: Paired excretory tubes very prominent in most specimens, each running laterally from anal vesicle to end blindly in basal segment of first leg.

Larval mites on which these observations were made were associated with adults in 2 dogs that were admitted to the Oklahoma State University, College of Veterinary Medicine Clinic. One dog, a 10-year-old female Pointer, was a patient for treatment of gunshot wounds of the head and thorax. During the initial treatment period mites were observed to crawl around on the external nares of the recumbent dog. Euthanasia was performed and at necropsy 120 mites were recovered from the nasal cavity and frontal sinuses; 91 were adults and 29 were larvae. The other dog, a 7-year-old male Pointer, died of distemper and meningo-encephalitis, and at necropsy 130 mites were taken from the nasal cavity and frontal sinuses; 98 were adults and 32 were larvae. Both dogs were natives of Payne County, Oklahoma.

An egg apparently of this mite was found among the mites collected from the second Pointer. This egg was oval and measured 615 by 538 microns. There was no debris on the shell and the internal mass appeared to be in an early stage of embryo development. The conditions which accounted for its presence, in the free state, were not determined. It could have been extruded naturally or displaced from a ruptured female. Two eggs were removed from intact females by dissection for comparison. They were oval with blunt ends, similar morphologically to the free egg and measured 569 by 397 microns and 494 by 419 microns, respectively.

Nymphs of this species have not been seen; however, the situation may be similar to that in *Pneumonyssus simicola,* in which both the protonymph and deutonymph stages apparently have a very short duration.

Numerous reports have appeared in the literature describing the appearance and distribution of this acariasis of the upper respiratory tract of dogs in the United States, Australia, and South Africa. *P. caninum* apparently inhabits only the nasal cavity and paranasal sinuses for it has not been found in the trachea, bronchi, or lungs. Although the life cycle of this mite is unknown, transmission is probably through direct contact for mites have been observed to crawl, in more than one instance, on the external nares of a recumbent host. Fully developed larvae have been observed in eggs in the abdomen of females and it appears that the mites are ovoviviparous. Eggs containing embryos in various stages of development can be seen readily in the abdomen of either live or preserved adult females. An egg takes up most of the abdomen because of its large size. This relationship, egg to adult female mite, is shown in a photomicrograph in an article by Koutz et al (1953).

Breed, age, and sex of a dog apparently do not have a direct relation to the dissemination of this mite. It appears to have a worldwide distribution and it has been determined that it may infest the following breeds of dogs: Airedale, Cocker Spaniel, Collie, Coonhound, Doberman, English Setter, German Shepherd, Great Dane, Greyhound, Laborador Retriever, Pointer, Ridgeback, and Terrier.

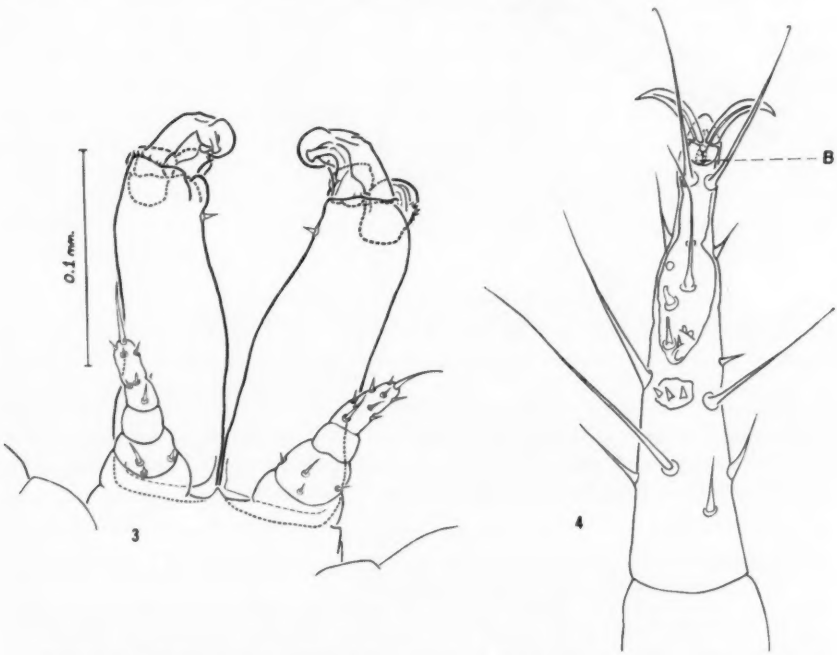

FIGURE 3. Chelicerae and palpi, adult male, *P. caninum,* dorsal view. ×430. Note the presence of a spinelet near the base of the chelae.

FIGURE 4. Tarsus I, larva, *P. caninum,* dorsal view. ×430. Note the arcuate hook on each of the paired terminal sensory setae. Caruncle is discernible. Letter "B" indicates the position of a short blunt process located on ventral surface opposite joint pieces in tarsal tip.

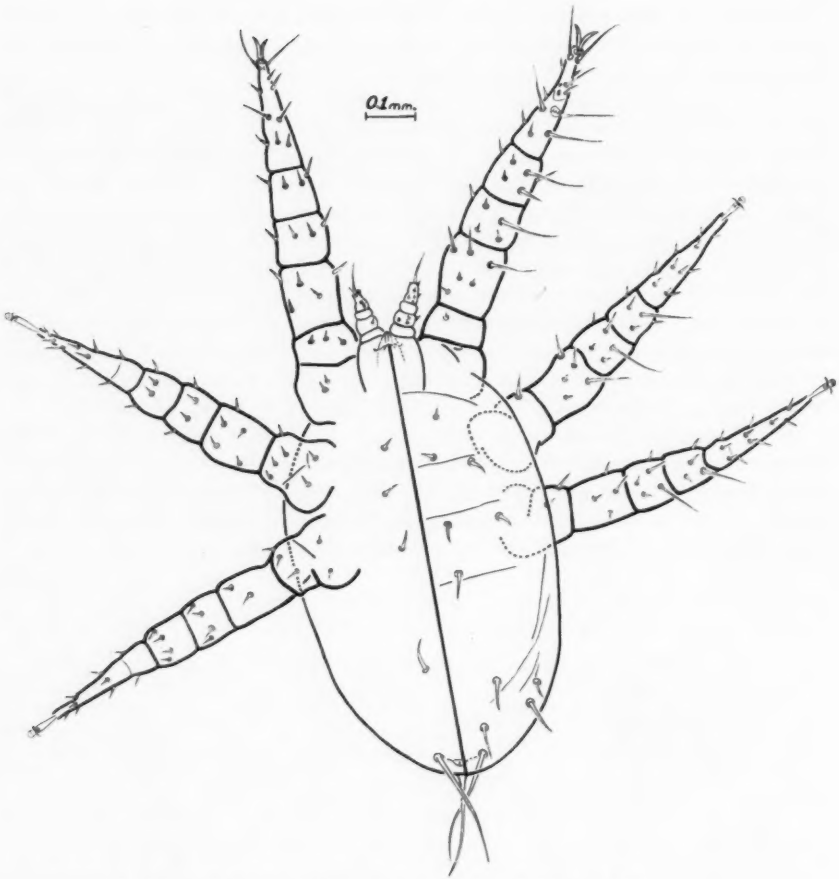

FIGURE 5. Larva, body, *P. caninum*, ventral and dorsal views. ×100.

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RECORDS AND NEW SYNONYMY OF NEW WORLD *HAEMAPHYSALIS* TICKS, WITH DESCRIPTIONS OF THE NYMPH AND LARVA OF *H. JUXTAKOCHI* COOLEY

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The genus *Haemaphysalis* Koch is poorly represented in the New World. Cooley (1946) recognized four species, *leporis-palustris* (Packard), 1869, *chordeilis* (Packard), 1869, *kochi* Aragão, 1908, and *juxtakochi* which he described as new. The only nomenclatural change since that time has been the substitution by Aragão and Fonseca (1951) of the name *kohlsi* for *kochi* because of long overlooked preoccupation of the latter name by *H. concinna* var. *kochi* Neumann, 1905. In the present paper, additional distributional and host data are provided, *kohlsi* is reduced to a synonym of *juxtakochi*, and the nymph and larva of the latter are described for the first time.

The collections examined were received from various individuals and institutions. Those of the Chicago Natural History Museum (CNHM) were sent by Dr. R. L. Wenzel, and one from the American Museum of Natural History (AMNH) was received through the courtesy of Dr. W. J. Gertsch. Panama collections by P. Galindo, G. Hartmann, and L. C. Dunn were made available by Dr. G. B. Fairchild, Gorgas Memorial Laboratory, and others from Panama were sent by Major G. Field and R. M. Altman, U. S. Army. Dr. Pierre Fauran, Pasteur Institute, Cayenne, permitted me to examine the one nymph of *H. juxtakochi* recorded from French Guiana. Dr. Harry Hoogstraal, U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, supplied the illustrations which were prepared by R. Strekalovsky.

Haemaphysalis leporis-palustris (Packard)

As stated by Cooley (1946) the rabbit tick occurs from Alaska and Canada southward to Argentina. The most northerly records appear to be those of Philip et al (1954) from hares near Circle, Alaska. Data for Canada are given by Gregson (1956) who records it from British Columbia eastward to Newfoundland. The species occurs throughout the United States and records accumulated since 1946 have added little to what has already been known concerning its distribution and host relationships. However, there are so few published records from Mexico, Central America, and South America that available records are detailed below.

MEXICO. *Recorded:* Without locality or host (Neumann, 1897); Tamaulipas, off groove-billed ani, 1 nymph, and Nuevo Leon, off small sparrow, 1 larva, probably this species, and Durango, without host (Hooker, Bishopp and Wood, 1912); said to be found frequently on rabbits and hares throughout the country (Chavarria, 1941); without new distributional or host data (Cooley, 1946; Vargas, 1955).

Examined: RML 17472, Santiago, Nuevo Leon, "off rabbit," June 20, 1940, 5 males, 11 females (H. Hoogstraal); RML 21622, Bravo, Nuevo Leon, off "hare," March 14, 1945, 2 males (R. Tovar); RML 14004, San Dimas, Durango, off "gray squirrel," several adults,

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nymphs, and larvae, Nov. 27, 1937 (E. B. Palmer); RML 14010, locality as above, off "rabbit," Nov. 25, 1937, several larvae and nymphs (E. B. Palmer).

GUATEMALA. *No previous records.*

Examined: RML 31387, Finca San Rafael, Sacatepequez, off *Sylvilagus floridanus chiapensis*, July 1, 1948, 1 female (R. D. Mitchell and Luis de la Torre, CNHM).

PANAMA. *Recorded:* Matachin, Canal Zone, off *"Dasyprocta* sp. probably *punctata isthmica,"* 1911, stage and number not stated (Nuttall and Warburton, 1915; Dunn, 1923); specimens off a rabbit in laboratory stock that had arrived from the United States about 2 months previously (Dunn, 1923).

Examined: RML 33497, Summit, Canal Zone, off *Sylvilagus gabbi* (probably), Aug. 3, 1954, 3 females; Gamboa, Canal Zone, host as above, April 19, 1955, 1 female, 1 nymph; Canal Zone, 1955, several larvae and nymphs from unnamed birds (all by Major G. Field, USA). RML 34923, Rio Mindinga, San Blas, off *Sylvilagus brasiliensis*, May 22, 1957, 2 females (P. Galindo); RML 34924, same locality, off *Sylvilagus* sp., May 29, 1957, 6 females, 3 nymphs, 1 larva (P. Galindo).

COLOMBIA. *Recorded:* Muzo, Boyaca, off "wild rabbit," adults and nymphs, also a nymph from man; Casablanca, Girardot, Cundinamarca, off "wild rabbit," adults and nymphs (Osorno-Mesa, 1941).

Examined: RML 31423, Socorro, upper Rio Sinu, Bolivar, off *Metachirus nudicaudata*, March 20, 1949, 1 nymph (P. Hershkovitz, CNHM).

VENEZUELA. *Recorded:* Caicara del Orinoco, Bolivar, off *Oryctolagus* sp., July 7, 1947, 7 males, 6 females (Vogelsang and Santos Dias, 1953).

Examined: RML 28927, State of Aragua, off *Sylvilagus floridanus valenciae*, Aug. 1, 1950, 2 males, 1 female (E. Schwarz).

PERU. *No previous records.*

Examined: RML 25229 and RML 25234, Siclamache, Huancabamba, Piura, off "wild rabbit," Nov. 1946 and Oct. 1947, 2 females and 1 male, 2 females and 1 nymph respectively (A. Macchiavello); 33576, Canchaque, Huanchabamba, Piura, off *Sylvilagus brasiliensis*, May 18, 1954, 1 nymph (C. Kalinowski, CNHM); RML 33577, Matapalo, Tumbes, off *Proechimys guyannensis*, July 2, 1954, 1 nymph (C. Kalinowski, CNHM).

BRAZIL. *Recorded:* Without host or locality, 1 female (Neumann, 1901); Manguinhos, Federal District, Xerem, Rio, and Bauru, São Paulo, adults off *Lepus brasiliensis* and domestic rabbit, nymphs off *Dasyprocta* sp. (Rohr, 1910); same data (Aragão, 1911); Annapolis, Goyaz (Aragão, 1938).

Examined: State of Rio, off *Sylvilagus minensis*, 1 male, 1 female, no other data.

PARAGUAY. *Recorded:* San Bernardino, female, nymph, host not stated (Nuttall and Warburton, 1915).

Examined: RML 31688, Yhu, off *Dasyprocta paraguayensis*, Mar. 8, 1950, 1 male (M. Hertig).

ARGENTINA. *Recorded:* Colonia Mocovi, Santa Fe, off *Penelope obscura*, September, and also said to attack man (Lahille, 1905); Salta, host not specified (Aragão, 1936); Campo Durand, Salta and El Siambon, Tucuman, off *Sylvilagus* sp. 1 male, 3 females, 7 nymphs (Aragão, 1938); locality and host as above (Boero, 1945).

Haemaphysalis juxtakochi Cooley

Haemaphysalis kochi Aragão, 1908
Haemaphysalis juxtakochi Cooley, 1946
Haemaphysalis kohlsi Aragão and Fonseca, 1951 (new name for *H. kochi* Aragão preoccupied by *H. concinna* var. *kochi* Neumann, 1905). *New synonymy.*

Study of the specimens identified by Cooley (1946) as *kochi* and *juxtakochi* has shown that the two species are the same. Cooley separated the two primarily on differences in the hypostomal dentition which is now found to be variable. He stated that it was 5/5 in *kochi* and 4/4 in *juxtakochi* though he figured it as 4/4 for the *kochi* male. The original description of *kochi* states that it is 4/4 in the male and 5/5 in the female. The male and two females determined by Aragão as *kochi* from *Mazama* sp., Pernambuco, Brazil, and accepted by Cooley as this species have the teeth so arranged. However, of the 9 females and 7 males from Venezuela identified by Cooley as *kochi* all have the dentition 5/5 except 1 female in which it is 4/4½ and 3 males in which it is 4½/4½.

All specimens determined by Cooley as *juxtakochi* still remaining in the RML

collection, including the holotype and allotype, have 4/4 dentition in both sexes except 1 female in lot 21585 from Panama in which it is 4½/4½. Similarly it is 4/4 in all others I have seen except in a female from Mexico, RML 25555, where it is 4/4½ and in 2 of 3 females from Panama, RML 34925, where it is 4½/4½ and 4½/5, respectively.

The differences noted by Cooley in the coxal spurs of his *kochi* and *juxtakochi* are likewise variable and are of no specific value.

Published records additional to those given by Cooley (1946) who recorded this species from Panama, Venezuela, and Brazil, and collections I have seen since then, are listed below.

MEXICO. *No previous records.*

Examined: RML 25555, near Limon, Tamaulipas, host not stated, Aug. 20, 1947, 1 female (C. and M. Goodnight, AMNH); RML 23777, Ocozocoatla, Chiapas, off *Mazama sartorii*, Dec. 13, 1946, 1 male (Miguel Alvarez del Toro).

PANAMA.

Examined: RML 19852, Alajuela, Canal Zone, off *Odocoileus*, Jan. 12, 1934, 4 males, 1 female, 1 nymph, 1 larva (L. H. Dunn); RML 34925, Rio Candela, Chiriqui, off *Mazama*, Feb. 21, 1955, 3 females (G. Hartmann); RML 35190, Ft. Kobbe, Canal Zone, off "deer," May 10, 1959, 3 females (R. M. Altman); RML 35191, locality and host as above, May 17, 1959, 1 male 3 females (R. M. Altman).

COLOMBIA. *No previous records.*

Examined: RML 34868, La Macarena, Rio Guapaya, Meta, off *Mazama*, Mar. 15, 1957, 1 female; RML 34843, locality as above, off *Mazama americana*, Mar. 14, 1957, 1 nymph; RML 34854, locality and host as above, Apr. 7, 1957, 1 nymph; RML 34858, San Juan de Arama, Los Micos, off *Tapirus*, Feb. 22, 1957, 1 nymph (all by K. von Sneidern, CNHM).

TRINIDAD. *No previous records.*

Examined: RML 33141, Cumaca, off *"Mazama rufa* or *Coelogenys paca,"* June 24, 1954, 7 males, 9 females (T. H. G. Aitken, Trinidad Regional Virus Laboratory).

VENEZUELA. *Recorded:* Calabozo and Piritu, Guarico and Maracay, Aragua, off *Mazama* sp. and *Odocoileus* sp. (Vogelsang and Cordero, 1940).

FRENCH GUIANA. *Recorded:* Saut Tigre, Territoire de l'Inini, off "antilope" (Floch and Abonnenc, 1940); Oyapock, Cafesoca, off man, Dec. 2, 1939, 1 nymph (examined); same locality, off *Mazama americana*, June 7, 1946, 2 females; Saut Tigre, off host as above, Jan. 20, 1940, 1 male, 1 female; Compte, Menado, off *Tapirus terrestris*, Jan. 14, 1942, 6 males, 2 females; Approuague, off *Mazama americana*, Apr. 10, 1944, 5 males, 1 female; Haute-Mana, off host as above, Mar. 14, 1944, 13 males, 2 females; Tonnégrande, off *Tapirus terrestris*, Feb. 29, 1955, 1 male (Floch and Fauran, 1958).

BRAZIL. *Recorded:* Baurú, São Paulo, off *Cervus campestris*, Nov. 15, 1907, 1 male, 2 females, and Jacutinga, São Paulo, Apr. 1907, 1 male (Aragão, 1908); Rio Guapore, Matto Grosso, off deer (Aragão, 1911); above localities plus Tapera, Pernambuco and Sacramento, Minas Gerais, off *Mazama* sp. and *Cervus paludosus* (Aragão, 1936).

URUGUAY. *Recorded:* Department of Artigas, off *Odocoileus campestris* July 1922 (Vogelsang and Cordero, 1939).

ARGENTINA. *Recorded:* Jujuy, off *Mazama* sp., 2 males, 1 nymph (Aragão, 1935); El Siambon, Tucuman, off cow, 1 female (Aragão, 1938); Diamante, Entre Rios, and El Siambon and El Portezuelo, Tucuman, and Reyes, Jujuy, off cow, goat, *Mazama simplicicornis, M. rufa*, and dog (Boero, 1944, 1945). Boero (1959) states that although deer are the preferred hosts it adapts well to cattle and has been confused with the cattle tick, *Boophilus microplus*.

Description of Nymph and Larva

Specimens available consist of 1 nymph and 1 larva from *Odocoileus*, Panama, 3 nymphs from *Tapirus*, Colombia, and 1 nymph from man, French Guiana as detailed above.

The nymph (fig. 1) closely resembles that of *H. leporis-palustris*, even to the extent of having cornua on the basis capituli ventrally, but differs in the following respects. *Capitulum:* Ventral spur of palpal segment 3 longer and sharper; palpal segment 2 ventrally with 2 fine hairs on the internal margin as compared to 4 or more definitely stouter hairs in *leporis-palustris;* the ventral cornua vary from definite to faint, whereas these are consistently distinct in all *leporis-palustris* I have seen. *Scutum:* Notably more broadly rounded posteriorly; cervical grooves less nearly parallel. *Legs:* Coxal spurs like those of *leporis-palustris* but no external spur on coxa I; ventral spurs on trochanters I–III variable, poorly developed in specimens from Colombia and French Guiana but distinct in the one nymph from Panama.

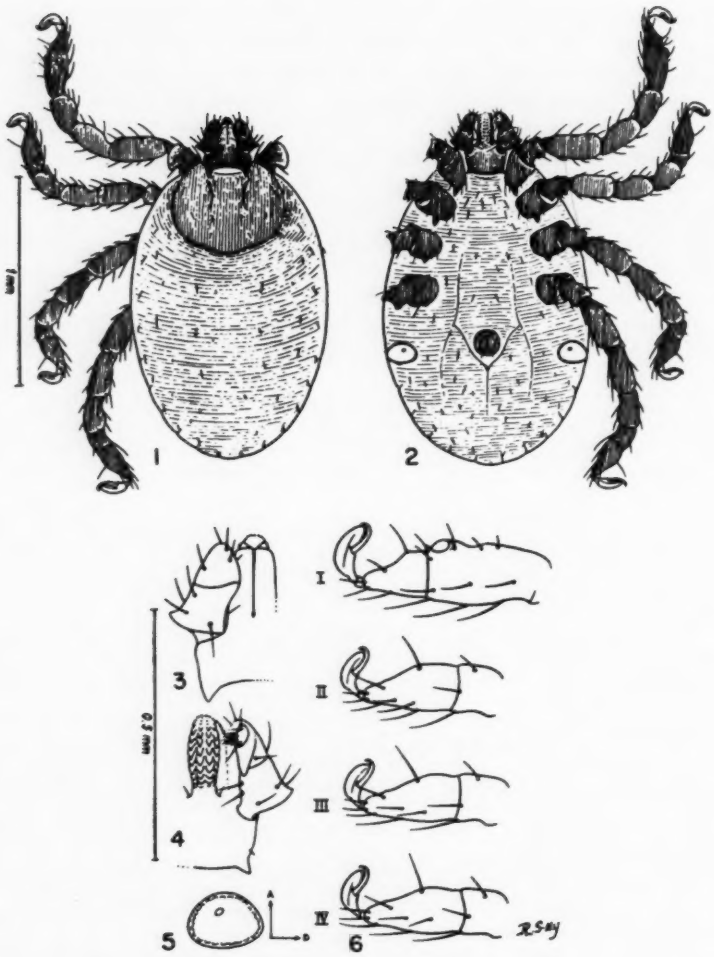

FIGURE 1. *Haemaphysalis juxtakochi*, nymph: 1, dorsal view; 2, ventral view; 3, capitulum, dorsal view; 4, capitulum, ventral view; 5, spiracular plate (A, anterior; D, dorsal); 6, tarsi I to IV.

The larva (fig. 2) too is very similar to that of *leporis-palustris*, but appears to be distinguishable by the absence of cornua on the basis capituli dorsally, the relatively broader scutum (about 0.24 mm long by 0.37 mm wide vs 0.25 mm long by 0.30 mm wide in *leporis-palustris*) and the somewhat longer and sharper spur on coxa I.

Haemaphysalis chordeilis (Packard)

This species, sometimes referred to as *H. cinnabarina* Koch, appears to be restricted to southern Canada and the United States although it has been recorded from St. Croix, Virgin Islands, from "Wilson Plover (*Charadrius wilsonia*)" (Beatty, 1944). Data for Canada are summarized by Gregson (1956) who refers to it as the common bird tick of Canada and states that it occurs abundantly on

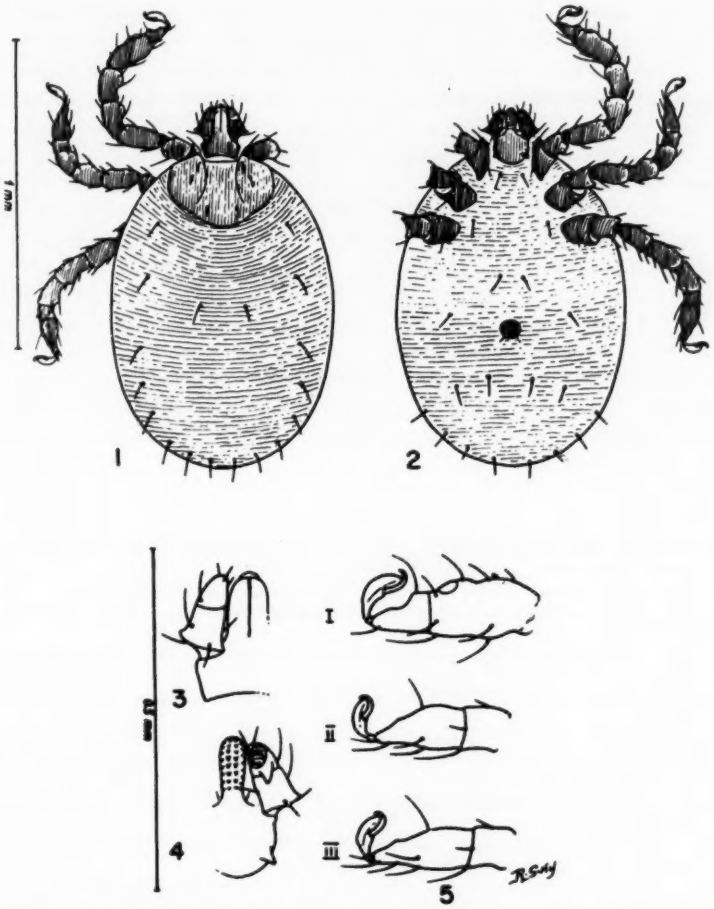

FIGURE 2. *Haemaphysalis juxtakochi*, larva: 1, dorsal view; 2, ventral view; 3, capitulum, dorsal view; 4, capitulum, ventral view; 5, tarsi I to III.

upland game birds except in the Pacific Coast wet belt. All known records for northeastern North America are given by Bequaert (1946) who states that the ruffed grouse is the chief host of all stages in this region. He also mentioned having seen specimens from spruce grouse in Oregon and from sharp-tailed grouse in North Dakota. McIntosh (1934) saw specimens from South Dakota from sharp-tailed grouse, September 25, 1933. Riley (1941) recorded it from game birds and young turkeys in Minnesota and Carpenter (1956) reported finding a male on vegetation, January 9, 1952, at Fort Barry, California. A female in the RML Collection, determined by F. C. Bishopp, is from cow, Fruitland, Washington, April 27, 1910 (Bishopp No. 1564).

SUMMARY

Host and distributional data for the three species of *Haemaphysalis* occurring

in the New World, viz, *leporis-palustris* (Packard) 1869), *juxtakochi* Cooley, 1946, and *chordeilis* (Packard), 1869, are presented.

H. leporis-palustris occurs principally on lagomorphs and birds from Alaska to Argentina. Because there are so few published records of this species except for Canada and the United States, all available for Mexico, Central America, and South America are given. The species is recorded for the first time from Guatemala and Peru.

H. kohlsi Aragão and Fonseca, 1951 (new name proposed for *H. kochi* Aragão, 1908, preoccupied by *H. concinna* var. *kochi* Neumann, 1905), is reduced to a synonym of *H. juxtakochi* Cooley, 1946. The nymph and larva of the latter are described and figured. Deer are the principal native hosts of adults and presumably of the immature stages also. The species is recorded for the first time from Mexico, Colombia, and Trinidad, and data for Venezuela, French Guiana, Brazil, Uruguay and Argentina where it has been previously recorded are given.

Published data pertaining to *H. chordeilis*, the bird tick, are briefly reviewed. All known records are from southern Canada and the United States except one from St. Croix, Virgin Islands.

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MICRO-ANATOMY OF THE PROVENTRICULUS OF THE COMMON RAT FLEA *XENOPSYLLA CHEOPIS* (ROTHSCHILD)

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That the flea is the vector responsible for the spread of plague was suggested by early workers such as Ogata (1897), Simond (1898), Verjbitski (1908), Gauthier and Raybaud (1902–3), Tidswell (1903) and many others. Liston (1905) clearly showed that *"Pulex cheopis"* (*Xenopsylla cheopis*) in Bombay readily attacked man during the plague season. Since then, through the laborious investigations of the English Plague Research Commission of India (Advisory Committee) (1906) and many other workers, it was shown that the disease is transmitted especially among the rodents by the bite of an infected flea in which regurgitation of the blood due to obstruction (blocking) of the proventriculus introduces the plague bacilli into the body of the host. This blocking according to Bacot and Martin (1914) is caused by a rapid multiplication of the bacilli which eventually fill up the interspaces between the spinous processes which project into the lumen of the organ. Quoting Eskey (1938), *"Xenopsylla cheopis* were found to be much more susceptible to plague infection than any of the other fleas tested . . . it would appear that *cheopis* develop blockage earlier and more readily than do other fleas which would tend to make them much more dangerous vectors of plague".

Though much has been added to our knowledge of the anatomy of the fleas since the earliest publication of "Anatomie des Hundeflohes" by L. Landois (1866), no detailed account regarding the internal structure of the proventriculus has hitherto been available. The present work was therefore undertaken with a view to elucidating the morphology and the physiological significance of the various micro-anatomical structures which constitute this organ. As the main purpose of this paper is to give an account of these structures only, and as the same is based on a micro-anatomical background, it would be impertinent on the part of the author to claim this small piece of work as a comprehensive one. It is however hoped that it will serve as an aid to those investigators who are engaged in the studies of biological correlation between the plague bacilli and their flea-host.

MATERIALS AND TECHNIQUE

Living specimens were obtained from an extremely flourishing culture maintained by adopting Leeson's method (1932), and the insects were anaesthetized and dissected in physiologically normal saline for observing living movements of the proventricular walls. Proventriculi were also teased out in normal saline for studying the spines. Mounting of freshly teased out spines in the DeFaure's fluid tinted light pink with borax carmine yielded excellent results. Different fixatives were tried but Carnoy II was found to be most satisfactory. Double embedding (celloidin and paraffin wax) method was employed for cutting serial sections. Delafield's haematoxylin and eosin were mostly used for staining. The "blow and roll" technique devised by the author and described below, proved to be of a great help for the study of the entire surface anatomy of the organ, including the arrangement, pattern and distribution of the spine bases, and for calculating the total number of proventricular spines in both the sexes.

Freshly dissected out proventriculi were allowed to float freely in normal saline contained

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by a depression block, and after mounting and focusing the same under the low power of the microscope, an extremely slow and steady jet of air was blown against them. By doing so, different parts of each organ rolling and shifting in the saline could be brought into view. A critical examination of the organ in any desired position was thus facilitated (fig. 1). It also became possible to obtain microphotographs of the organ in various positions by the same method.

DESCRIPTION

The proventriculus is a globular portion of the alimentary canal situated just before the stomach in the first abdominal segment of both sexes (fig. 2). Its anterior region tapers till it becomes continuous with the oesophagus. Though normally there is no distinct separation between this anterior region and the oesophagus, the posterior limits of the latter in many specimens were observed to be marked externally by a circumferential fold (fig. 4). The whole organ is divided into the following three regions:

(1) THE ANTERIOR SPINELESS REGION (fig. 4, Ant.). Though Faasch (1935) has applied the term "atrium" to this region and described it as a mere posterior portion of the oesophagus, its structural peculiarities suggest that it is a part of the proventriculus itself. The intima of this region lies over a flat epithelium, and both of these when not stretched by the blood contents, are thrown into 15 low ridges or folds projecting into the lumen and gradually increasing in depth in the second spine-bearing region that follows. The epithelial cells which rest over a basement membrane appear to be longer than broad and the fibres of muscular sheath resemble those of the oesophagus in general structure and arrangement. The circular muscles surround the longtitudinal ones, but the latter are divided into well separated groups lying in the spaces of the folds described above.

(2) THE MIDDLE SPINE-BEARING REGION (fig. 4, Mid.). The intima of this region is dense, folded and closely beset with 15 consecutive rows of backwardly directed chitinous spine-like bristles in the female, and 12 similar rows (with negligible variations) in the male. Each row consists of 30 spines in the female (fig. 3), and 22 (with negligible variations) in the male. Thus there are altogether 450 spines in the female and 264 in the male. The folds of the intima are continuous with those of the anterior spineless region described above. The color of this region ranges from light amber to dark brown according to the nature and quantity of blood it contains. Its size and to some extent its shape also vary slightly for similar reasons. It is broader than long, and when its walls are not subjected to muscular contraction, it measures on an average 105 microns by 231 microns in the female and 84 microns by 168 microns in the male. Though a greater part of its lumen is occupied by the spines, there still remains a clear, central circular passage (figs. 3 and 6, Sp) for the blood to pass through, whose average diameter is 84 microns in the female and 63 microns in the male. The epithelium resting over a basement membrane differs in appearance from that of the anterior spineless region, and the protoplasm of the cells together with the nucleus projects into the cavity of the hollow spines. The muscular sheath is very well developed and consists of the usual circular fibres surrounding the longitudinal ones. The former form thick bands closely packed together, while the latter are continuous with the longitudinal muscle strands of the anterior spineless region. Patton and Cragg (1913) state: "There are no musculatures whatsoever attached either to the oesophagus or to the proventriculus." They have described the closure of the lumen of the latter as being "probably

due to the negative pressure produced by the contraction of the musculatures of the pharynx." This appears to be obscure not only in the light of the above description but also in view of the fact that even Landois (1866) in his earliest work has shown the presence of these muscles in the dog flea. Faasch (1935) has further given a very clear account regarding the function of these muscles.

(3) THE POSTERIOR SPINELESS REGION (fig. 1A, Post.). This constitutes the inner wall of the so-called stomodaeal valve formed by the telescoping of the fore-gut into the mid-gut. The valve itself is double walled, both the walls being adnate. The epithelium of the inner wall is cuboidal and is lined internally by intima, while that of the external wall consists of narrow elongate cells with no lining of intima, and continuous with the epithelial cells of the mid-gut. The shape of this region ranges from cylindrical to cylindroconical according as it is subjected to the muscular contraction of its walls. The muscular sheath resembles more or less that of the middle spine-bearing region in structure and general appearance. Though according to Snodgrass (1944) the so-called stomodaeal valve acts as a barrier to regurgitation from the stomach, it is doubtful whether this well developed double walled structure strengthened by chitinous intima would really have a valvular action. This very doubt which has been held by Wigglesworth (1950) has also been endorsed by Pradhan (1936) and others. Further details regarding the function of this structure are discussed in the portion to follow.

Description and Micromeasurements of the Spines

In cleared and unstained preparations the outer surface of the middle spine-bearing region appears to be studded mostly with hexagonal areas (fig. 5) which have been only generally described by Landois (1866) as "polygonal plates from each of which a chitinous bristle springs into the lumen of the organ." The pattern is actually formed by the bases of the chitinous processes of the intima immediately contacting the underlying epithelial cells. These processes have been described as circular or polygonal in cross section by several workers. Patton and Craig (1913) have mistakenly attributed the polygonal shape to the spines being "closely compressed together at the base", and Bacot and Martin (1914) have described them as tooth-like epithelial cells. However, the fact that the spines vary in shape, size, structure and rigidity according to their distribution on the intima, has been completely overlooked. These chitinous processes or spines are slender hollow unicellular outgrowths of the intima with a nucleated plasmatic core originating from the underlying epithelial cells, and their bases, according to their shape, present various sculptured designs on the intima. Meridionally the spines of the alternate rows being immediately one behind the other, a vertical section (fig. 5C. ab, cd) of the proventricular wall would either pass through only 7 or 8 such spines at a time. This led Bacot and Martin (1914) and Waterston and Jordan (1942) to conclude that "there are seven rows of spines in *X. cheopis.*" Further, no mention has been made by any of the previous workers regarding the difference in the number of these spines in the two sexes of this insect.

The spines of the first 2 rows in the female are small, weakly sclerotized papilla-like protuberances, more lobular than spine-like, and more or less resemble in shape the lobular lips guarding the orifice between the honey-stomach and the proventriculus of the bees. Each spine of the 1st row is bluntly rounded at its tip

and has a somewhat circular base. The tip of the spine of the 2d row is bilobed and the circular margin of its base is slightly depressed in the front (fig. 9). Each spine of the 3d row is notched at its extremity and thereby produced into a pair of stiff and sharply pointed processes. The shape of its base indicates a gradual transition from that of the spines of the 2d row to the shape of the spine-bases of the 4th row. The spines of the next 5 rows (4th, 5th, 6th, 7th, and 8th) are stouter, more thickly sclerotized and similarly notched and thereby double-pointed at their extremities. Their bases however are hexagonal in shape and tangentially elongated. The bases of the stouter spines of the 9th and the 10th rows are roughly hexagonal, and the aforedescribed notch in the tip of each is completely filled by an extremely thin and scarcely perceptible transparent sclerous membrane. Due to this their bifid tips are transformed into truncated ones. The spines of the 11th and the 12th rows are the stoutest of all and have their hexagonal bases stretched out meridionally. They are spatulate in shape and are distinctly truncated at their converging extremities. In profile they appear to be sabre-shaped (fig. 7). The bases of the spines of the 13th row are similar in shape to those of the preceding row but are smaller in size. Their rigid tips are sharply pointed and slightly recurved. They also appear to be sabre-shaped in profile. The tips of the spines of the 14th row are similar to those of the preceding row, but their bases are more or less elliptical. Finally, the short conical spines of the 15th row have circular bases.

Thus there are altogether 4 principal sets of spines in the proventriculus of the female, viz., (1) the first 2 rows of very thinly sclerotized lobular spines with bluntly rounded tips, (2) 6 rows of spines converging at their extremities into a pair of stiff and sharply pointed processes, (3) 4 rows of acutely bent and strongly sclerotized spines with truncated tips, and, (4) 3 rows of stouter and more acutely bent spines converging into rigid and sharply pointed recurved tips (fig. 9).

Deoras and Joshi (1958), however, in their very recently published abstract have noticed the occurrence of "four distinct rows of teeth with their converging pointed ends in the centre of the stomach". This appears to be rather ambiguous in view of the features described above. Moreover, the fact that the backwardly projected extremities of the spines are well enclosed within the aforedescribed posterior spineless region (the so-called stomodaeal valve) of the proventriculus was not noted. The extremities of the spines in any case do not appear to be projecting beyond the posterior limits of this region nor entering as far back as the centre of the stomach (fig. 6).

The proventriculus of the male is smaller in dimensions than that of the female, the spines in the former are proportionately smaller though identical in structure and distribution to those of the latter. However, the first 2 rows of spines and the last row in the female appear to be lacking in the male.

DISCUSSION

In the light of the above description it becomes clear that there is a progressive increase in length as well as bending of the spines with a gradual increase in the diameter of the spine-bearing region of the proventriculus, and that the spines arising from the main bulge of the region are all about equal in length, viz., 70 microns maximum (fig. 9). They then gradually decrease in length and bend more acutely with the decrease in diameter towards the posterior limits of the region. This results

in a clear central space of nearly even diameter for a direct and an uninterrupted flow of blood towards the mid-gut during the process of feeding. That a contrivance like this enables the flea to feed rapidly when blood is available in abundance and to accomplish more leisurely the digestive process at a later stage may be verified by opening out the stomach of a freshly fed flea in physiologically normal saline, when it will be observed that the blood cells are but little affected by the spines during the actual process of feeding. It has also been pointed out by Faasch (1935) and Wigglesworth (1950) that the spines break up the corpuscles subsequently during the process of digestion.

The mechanical trituration of the blood which follows into the mid-gut appears to be accomplished by several factors, viz., (1) the peristaltic movements of both the proventriculus and the mid-gut, (2) to and fro shifting movements of the proventriculus itself, and (3) the contractile action of the so-called stomodaeal valve.

Though the movements of the proventriculus and the mid-gut are independent of each other, the propulsion of blood into the former by the latter appears to be in perfect synchronization with the action of the spines. The peristalsis of the mid-gut takes the form of a series of discontinuous waves constantly changing its diameter at different portions, and the peristaltic and the antiperistaltic waves meet and merge periodically in the central region. Normally every 3 to 5 peristaltic waves are followed by an extremely rapid antiperistaltic one which forcibly thrusts the blood against the more acutely bent, sharply pointed spines projecting into the cavity of the posterior spineless region. The impact thus produced probably aids in fragmentation of the blood cells which then traverse further up and are probably taken over by the rigid spines situated in close apposition at a higher level. Trituration is then probably effected due to the adduction and subsequent grinding movements of these spines resulting from the continuous peristaltic and antiperistaltic movements brought about by the co-ordinated sequential contractions of the highly elastic circular muscle bands of the proventricular walls.

In this connection it may be that the function of each set of spines should unquestionably differ according to its morphology and position. In all probability the anterior half of the spine-bearing region ("proventriculus anterior" of Eidmann (1924)), with its convergent folds and moderately bent double pointed spines converging into its lumen, serves as an effective barrier to regurgitation, whereas the posterior half of the same ("proventriculus posterior") with deeper channels between the compact folds of its intima and more acutely bent, backwardly-directed stouter spines, aids in fragmentation and trituration of the blood cells, after permitting the entry of the ventricular fluid. In this respect the shape, structure, and disposition of the spines show little to suggest that "they act as strainers and detainers for the food entering the stomach" as stated by Deoras and Joshi (1958).

The pulsatile movements of the proventriculus (53 per minute on an average) were clearly observed for a long time (even for a couple of days in some cases) in slightly anaesthetized insects in freshly prepared physiologically normal saline. It was during this very observation that other interesting features with regard to this process were noted. The process of trituration described above was very often found to be accompanied by an extremely rapid squirting of the proventricular contents back into the mid-gut. This suggests that after the blood cells are triturated into particles of desired dimensions suitable for the action of the gastric enzymes, they are forcibly discharged into the mid-gut. This process is probably intensified by a

reduction in the calibre of the orifice of the posterior spineless region, in view of the fact that the shape of the so-called stomodaeal valve was observed to vary from cylindrical to cylindroconical in a large number of permanent preparations at hand. In the former case, the calibre of the orifice probably facilitates the entree of the blood into the proventriculus, while in the latter it acts as a spout for the discharge of blood under pressure exerted by the muscular walls of the organ. In this view the action of this so-called stomodaeal valve as a barrier to regurgitation from the stomach is rather doubtful, and it may be agreed with Wigglesworth (1950) that the same "is quite unsuited by its structure to act as a valve".

Another interesting feature frequently noted was that of the movements of some of the anterior spines of the spine-bearing region. Whether these movements were due to the force of the inrushing blood or took place under a specialized muscular control is a matter for further investigation before any conclusion could be justified.

Besides the activities of the proventriculus as detailed above, attention may further be drawn to its oscillating movements accompanied by periodic jerks. Faasch (1935) has amply demonstrated the presence not only of the muscles causing peristaltic and contractile movements of the organ, but has also described muscles extending from the proventricular walls right up to the beginning of the mid-gut. It is due to the action of these muscles that the entire organ according to him is shifted to and fro in an antero-posterior direction. This presumably produces a pounding effect on the blood as it is forcibly thrust against the sharply pointed and backwardly directed spines described above.

In the light of the above discussion it may be concluded that, apart from its primary function of preventing regurgitation, this remarkable antechamber of the ventriculus has many more functions to perform than are commonly assumed.

Though it cannot be within the scope of the present paper to discuss at length whether it is as a result of blocking of the proventriculus or by some other means that *X. cheopis* infected with plague bacilli eventually becomes infective, it may be pointed out that according to Douglas and Wheeler (1943) "the location of the bacterial plug is to some extent a characteristic of this species, and it is probably controlled by anatomical structures and physiological activities of the insect's alimentary tract". An attempt has been made in the present paper to explain the anatomical features but further results of a detailed study of the physiological alimentary activity should be awaited. Even the very aspect of the theory of regurgitation might probably need revision in the light of a recent and an outstanding publication on the anatomy of the head capsule of the dog-flea, *Ctenocephalus canis* (Curt.) by P. Wenk (1953) wherein the detailed structure of a posterior (postcerebral) pump has been described for the first time. This part of the stomodaeal section which has escaped detection in the past years is also present in the other fleas together with the usual cibarial and anterior (precerebral) pharyngeal pumps already described by Karandikar and Munshi (1949, 1950). The flea therefore has reached the greatest degree of specialization in that it presents combined dipterous features of Cyclorrhapha, Brachycera, and Nematocera, being singularly characterized by the possession of all the 3 pumps, the efficient working of which under special nervous controls produces a very effective, continuous and uninterrupted flow of blood towards the mid-gut. The mechanism of regurgitation under the circumstances still remains to be explained.

According to Deoras and Joshi (1958), "the complexity of teeth and their

greater number in the female point out that probably the female of the species (*X. cheopis*) is a better transmitter." Again, according to their own description, the maximum number of spines, viz. 722, is found in the female of *Ctenocephalides canis;* the next highest number, 554 and 429, in the females of *X. astia* and *Ctenocephalides felis,* respectively, and a comparatively smaller number, 381, in the female of *X. cheopis.* Possession of a greater number of proventricular spines, therefore, cannot be taken as a criterion for capability of better transmission. In this context it is worthwhile calling attention to the laborious experiments of Goyle (1927) whose results show that "the males of *X. astia* and *X. cheopis* transmit the plague bacilli more readily than the respective female species." Goyle's experiments have further confirmed those of Hirst (1923), and the mixed flea experiments of Webster and Chitre (1930) have clearly shown that "the males of *X. cheopis* and *X. brasiliensis* are much the most regular transmitters." Finally, it may be pointed out that workers such as Blanc and Baltazard (1942) have discredited the importance of this blocking mechanism in fleas, believing the chief factor in the flea's ability to transmit the infection to be the rapid multiplication of the plague bacilli in the fresh blood taken up in frequent feeding.

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EXPLANATION OF PLATES

PLATE I

FIGURE 1. Various positions and different surface views attained by the "blow and roll" technique. A, lateral view; B, basal view; C, frontal view. Ant., anterior spineless region; Mid., middle spine-bearing region; Post., posterior spineless region.

FIGURE 2. Photomicrograph, *Xenopsylla cheopis* female. P., proventriculus.

FIGURE 3. Diagrammatic median transverse section through proventriculus of *Xenopsylla cheopis* female showing row of 30 spines enclosing a central space.

FIGURE 4. Proventriculus with adjacent portion of esophagus and cardiac end of the ventriculus. F., circumferential fold anterior to the proventriculus; Oes., oesophagus; St., stomach.

PLATE II

FIGURE 5. Diagram illustrating sculptured pattern formed by the spine bases; strip of the proventricular wall B removed from A and flattened out as shown in C. Dotted lines ab, cd, represent vertical sections of the proventricular wall passing through 7 and 8 spine bases, respectively.

FIGURE 6. Camera lucida sketch of a median longitudinal section through proventriculus of *Xenopsylla cheopis* female showing arrangement and distribution of the spines. St, cardiac end of the stomach; V, stomodaeal valve.

FIGURE 7. One side of section illustrated in FIGURE 6 further magnified and drawn to scale. Nos. 1–15, sectional views of spines with nucleated plasmatic core.

FIGURE 8. Diagram illustrating the external structure of typical spines met with in proventriculus of *Xenopsylla cheopis* female. 1 to 2, weakly sclerotized spines; 3 to 8, spines with double pointed extremities; 9 to 12, spines with truncated tips; 13 to 15, spines with sharply pointed tips.

PLATE III

FIGURE 9. Description and micromeasurements of proventicular spines of *Xenopsylla cheopis.*

PLATE I

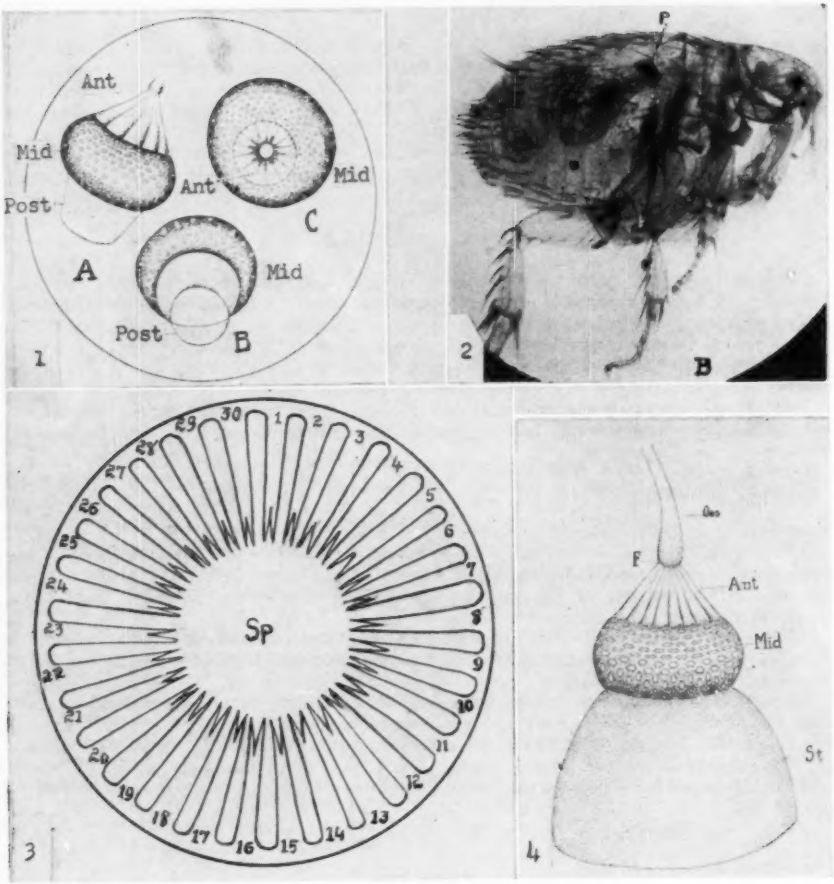

PLATE II

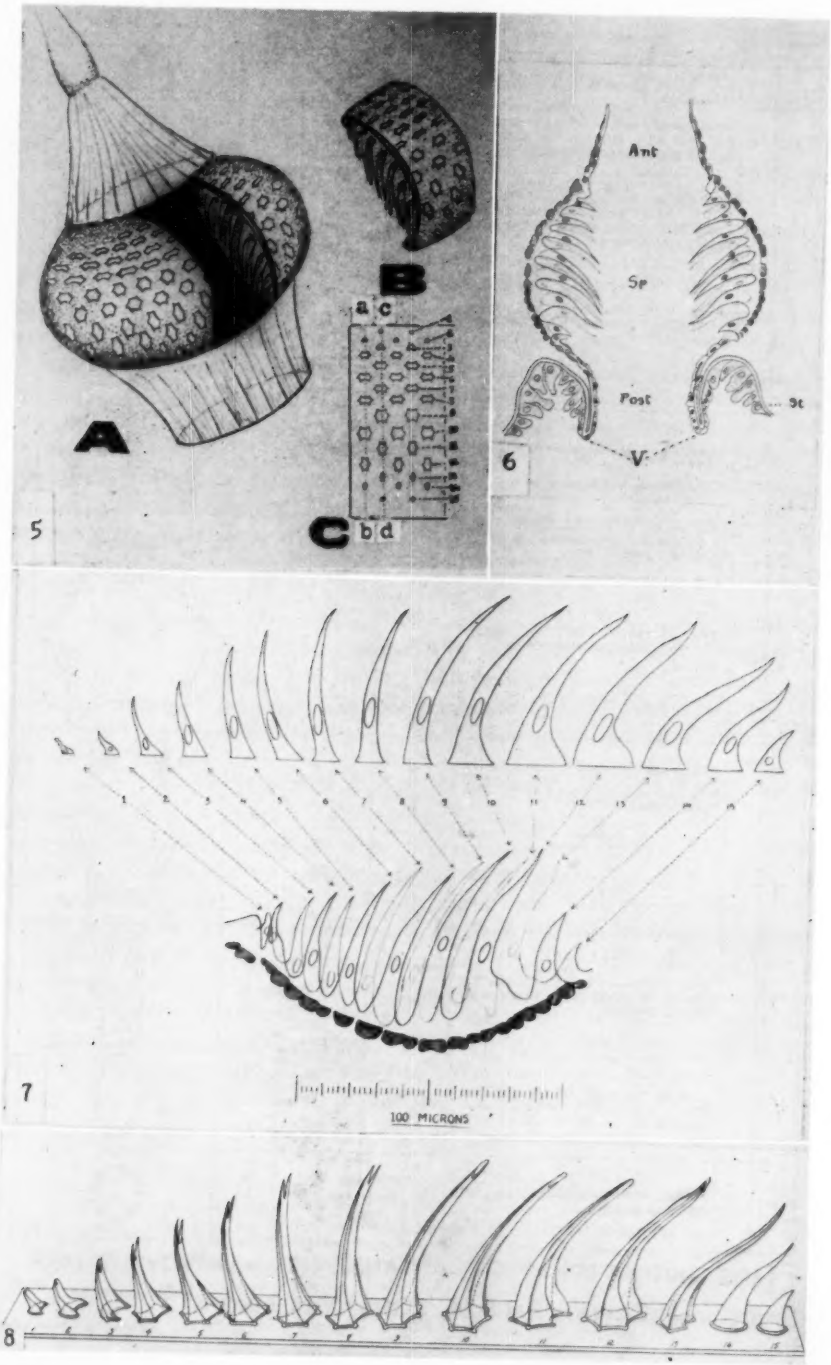

PLATE III

Row Number	Number of the Spines	Description of the Spines	Measurements in Microns.				Shape of the Tip	Shape of the Base.
			Height	Base	Notch	Nucleus		
1	30	BLUNT AND THINLY CHITINIZED PROTUBERANCES WITH BLUNTLY ROUNDED TIPS. BASES CIRCULAR WITH ANTERIOR DEPRESSION	6x6	6x6		3x3		
2	"	MORE OR LESS SIMILAR TO THE SPINES OF THE FIRST ROW, BUT THE TIPS BILOBED DUE TO A MEDIAN DEPRESSION.	10	8x8		3x3		
3	"	TIPS BIFURCATED DUE TO A MEDIAN NOTCH. BASES SHOW A TRANSITIONAL STAGE BETWEEN THE CIRCULAR AND THE HEXAGONAL SHAPES.	20	10x20	10x4	6x3		
4	"	SPINES WITH BIFURCATED TIPS AND TANGENTIALLY ELONGATED HEXAGONAL BASES.	30	10x25	10x4	8x4		
5	"	"	40	10x30	10x4	10x4		
6	"	"	50	10x30	10x4	12x4		
7	"	"	60	10x30	10x4	12x4		
8	"	"	70	10x30	10x4	12x4		
9	"	SAME AS ABOVE, BUT THE NOTCH FILLED UP WITH THIN CHITIN, GIVING THE TIP TRUNCATED APPEARANCE.	70	10x30	—	12x4		
10	"	SAME AS ABOVE, BUT THE BASES BECOMING BROADER MERIDIONALLY.	70	20x30	—	12x4		
11	"	STOUT BLADE-LIKE SPINES WITH TRUNCATED TIPS, AND BASES MERIDIONALLY ELONGATED AND TANGENTIALLY REDUCED.	70	25x20	—	12x4		
12	"		70	25x20	—	12x4		
13	"	STOUT BLADE-LIKE SPINES WITH SHARPLY POINTED RECURVED TIPS. BASES MORE OR LESS RHOMBIC.	70	16x10	—	10x4		
14	"	SAME AS THE SPINES OF THE THIRTEENTH ROW, BUT SHORTER IN SIZE. BASES MORE OR LESS OVOID.	50	13x8	—	6x3		
15	"	SHARPLY POINTED CONICAL SPINES WITH CIRCULAR BASES.	20	10x 10	—	3x3		

TABLE SHOWING DESCRIPTION AND MEASUREMENTS OF THE PROVENTRICULAR SPINES OF THE COMMON RAT-FLEA. (XENOPSYLLA CHEOPIS) ♀

9

SCALE 0 5 10 MICRONS

FACTORS CONCERNED WITH THE EPIZOOTIOLOGY OF *CAPILLARIA HEPATICA* (BANCROFT, 1893) (NEMATODA) IN A POPULATION OF *PEROMYSCUS MANICULATUS* IN ALGONQUIN PARK, CANADA

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The liver-inhabiting nematode *Capillaria hepatica* (Bancroft, 1893) Travassos, 1915, although reported from a variety of animals including man, is primarily a parasite of rodents (Read, 1949; Lubinsky, 1956; Skrjabin et al, 1957). The worm occurs in rodents in Algonquin Park, Canada. The location of these worms in the liver, usually surrounded with connective tissue, limited the number of intact specimens available for morphological study. Those adults which were recovered fit the description of this species given by Baylis (1931). Presumably only one species of nematode is involved, since eggs from various hosts overlap in their range of measurements, and since embryos from such eggs infected a variety of rodents in various cross-infection experiments. The eggs will not embryonate unless they are liberated from the liver of the host and provided with proper temperature, oxygen tension, and moisture (Bancroft, 1893; Fain, 1953; Fülleborn, 1924; Luttermoser, 1938; Shorb, 1931).

How the eggs are released from the liver is one of the interesting enigmas of parasitology. According to Shorb (1931), some workers have found a few eggs in the feces of the infected host and others have not found any. Luttermoser (1938) found eggs in the feces of mice with massive infections, but never in the feces from rats. Fülleborn (1924), Luttermoser (1938), Pavlov (in Skrjabin et al, 1957), and Shorb (1931) agree that release of enough eggs to perpetuate the species only occurs after death of the host. Luttermoser (1938), Orlov (in Skrjabin et al, 1957), Pavlov (in Skrjabin et al, 1957), and Shorb (1931) demonstrated that viable eggs rarely resulted from the natural decomposition of the host. It is generally accepted that the infected host must be eaten either by one of its own kind or by a carnivore, or a scavenger, and that the eggs embryonate only after passing out in the feces (Fain, 1953; Fülleborn, 1924; Luttermoser, 1938; Shorb, 1931). Chabaud (1955) *a priori* found it difficult to conceive that this species is perpetuated simply by cannibalism because the eggs would be liberated in a restricted space.

Data concerning infections of various rodents with *C. hepatica,* and other parenteral parasites, in different years in Algonquin Park, together with experiments on the biology of this nematode, especially the effect of starvation on release of eggs from the liver, are reported herein. These data lead to an explanation of the mode of natural transmission in a local area.

MATERIALS AND METHODS

Most of the wild, small mammals were trapped in standard wood snap-back mouse traps set with a variety of baits, primarily various dried fruits or a mixture of peanut butter and rolled

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oats. A modified Sherman live trap, with similar baits, was used also. The livers of all mice were examined grossly, and all lesions were examined with the aid of the microscope. Generally, the presence of the characteristic 2-plugged eggs indicated an infection with *C. hepatica*. Encysted metacestodes and very small unidentified nematodes were encountered also.

Small pieces of liver infected with eggs of *C. hepatica* were taken from natural or laboratory infections in various rodents, and ground in a tissue grinder to release the unembryonated eggs from the connective tissue. This material was washed and sedimented repeatedly until the supernatant was clear. The egg-bearing sediment was kept in standard screw-cap specimen vials under 1 cm or less of approximately 0.5 percent formalin, which was aerated several times a week during embryonation. Poor embryonation occurred if the vial was too full of liquid, or if there were too many eggs, or in the presence of heavy bacterial growth. The egg-laden vials were kept at various temperatures.

Animals were fed embryonated eggs of *C. hepatica* either mixed into a food pellet or by stomach tube. Generally, massive doses of eggs were used, but when known dosages of eggs were desired the eggs were suspended in 40 to 50 percent glucose solution.

To detect if eggs were escaping from the liver, feces and/or intestinal contents from infected mice were concentrated either by straining through cheese cloth and then repeatedly washing and sedimenting, or by flotation in saturated sodium nitrate, or by use of the Tollman acid-ether technique. If only a little debris remained, then the total concentrate was examined, otherwise only a fraction was examined. Experiments following the feeding of non-embryonated eggs to rodents revealed that approximately 10 percent of the eggs which were fed were recovered subsequently by the acid-ether technique.

Most species of rodents which were kept for varying periods of time in the laboratory were fed pelleted commercial fox meal and sunflower seeds, irregularly augmented by various greens and fruits.

For starvation studies the food intake of laboratory-reared *Mus musculus* L., *Peromyscus maniculatus gracilis* (LeConte)**, and *P. leucopus noveboracensis* (Fischer) was restricted in various ways. In the first trials the mice were fed ad libitum for varying but restricted periods of time. In other trials the total amount of food that was consumed during a full day was measured, and on succeeding days this quantity was reduced by a known amount, which gave a more controlled loss of weight. In another regimen mice were fed ad libitum on a modified Naftalein low protein diet consisting of: 8 percent casein (vitamin free), 3 percent brewer's yeast, 76 percent sucrose, 7 percent commercial lard, 2 percent cod liver oil, and 4 percent salts mixture (containing 10.01 g sodium chloride, 29.93 g dipotassium acid phosphate, 26.92 g calcium acid phosphate, 6.62 g calcium lactate, 2.58 g ferric citrate, 0.07 g potassium iodide, 10.63 g magnesium sulphate, and 15.39 g of dibasic sodium phosphate).

INFECTION PATTERN IN MAMMALS FROM ALGONQUIN PARK

Animals, mostly small rodents and insectivores, were trapped in Algonquin Park and examined for parasites every summer of 1952 through 1957. At first they came from a variety of habitats and no effort was made to trap selectively. In 1956 and 1957 a more selective trapping program was followed. Some beaver (*Castor c. canadensis* Kuhl) and muskrat (*Ondatra z. zibethicus* (L.)) were examined also. Of the nearly 2000 rodents of 15 species which were examined, the following harbored *C. hepatica* one or more times: *Clethrionomys g. gapperi* (Vigors)***, *Microtus p. pennsylvanicus* (Ord)***, *Napaeozapus insignis algonquinensis* Prince***, *O. zibethicus, Peromyscus maniculatus,* and *Synaptomys c. cooperi* Baird***. The high incidence of infection in *P. maniculatus* and *C. gapperi* indicates that the former and possibly the latter are important in disseminating *C. hepatica* in Algonquin Park. Probably infections in the other hosts represent accidental infections. No infections with *C. hepatica* occurred in 333 *Tamias striatus griseus* Mearns, or in 53 *Tamiasciurus hudsonicus loquax* (Bangs). Apparently the former is naturally resistant since several attempts failed to infect this species in the laboratory. On the other hand *T. hudsonicus**** was fed eggs once and became infected.

** Host names from: Miller, G. S. and Kellogg, R. "List of North American Recent Mammals." U. S. Nat. Mus. Bull. 205 (1955).

*** New host records.

The metacestodes of *Taenia mustelae* Gmelin, *Paruterina rauschi* Freeman, and *Cladotaenia globifera* (Batsch) develop in rodents only following the ingestion of their respective eggs passed in the feces of weasels and mink, or owls, or hawks (Freeman, 1956, 1957, 1959). Therefore, the incidence of infection of rodents with these metacestodes may be used as an index of the frequency with which these rodents contact such feces. Eggs of cestodes harbored by carnivores will be shed more or less continuously, whereas eggs of *C. hepatica* will occur in the feces of a carnivore for a short time only after an infected mouse is eaten by a carnivore. Consequently, if contact with feces of carnivores is essential for the transmission of *C. hepatica,* the incidence of infection with this parasite should be similar to or less than that of metacestodes acquired from the feces of carnivores. The incidence of metacestodes and *C. hepatica* in *C. gapperi* and *P. maniculatus* during 1952 through 1955 revealed notable differences (table I).

TABLE I. *Incidence of certain helminths in two species of rodents in Algonquin Park, 1952 through 1955.*

Species	Number examined	Percent infected with			
		Capillaria hepatica	*Taenia mustelae*	*Paruterina rauschi*	*Cladotaenia globifera*
Clethrionomys gapperi	142	2.8	2.8	0.7	0.7
Peromyscus maniculatus	769	9.4	0.6	0.1	0.1

C. gapperi must be exposed to carnivore feces more frequently than *P. maniculatus,* since 4.2 percent of the former contained metacestodes whereas 0.8 percent of the latter did (table I). In contrast, it is to be noted that 9.4 percent of the *P. maniculatus* harbored *C. hepatica,* whereas only 2.8 percent of *C. gapperi* were so infected. Furthermore, the *P. maniculatus* population had about 12-fold more infections with *C. hepatica* than all metacestodes combined, which is in contrast to the condition in *C. gapperi* where the incidence of the nematode is less than that of all metacestodes combined. Therefore, carnivore feces cannot be the source of much of the infection with *C. hepatica* seen in the population of *P. maniculatus.* On the other hand, in the population of *C. gapperi,* carnivore feces and/or contact with *P. maniculatus* may be the source of infection with *C. hepatica.*

It seemed desirable, therefore, to compare the incidence of infection with *C. hepatica* in the years 1952 through 1955 with the density of the population of *P. maniculatus.* No particular effort was made to trap selectively for *P. maniculatus* during these years, as was done subsequently. Dr. J. B. Falls (1959) kindly supplied data, derived from standardized trapping techniques carried on in the same habitats each year, which gave a measure of the density of the population for these years expressed as the number of *P. maniculatus* caught per 10 traps per night (table II). Notable is the remarkable correlation between the percent of *P. maniculatus* infected with *C. hepatica* and the density of mice, either compared directly (columns B and D, table II) or expressed as percent increase or decrease of infection and population respectively from the previous year (columns C and E, table II). These data suggest that most infections of *P. maniculatus* are density dependent and come from within the population itself.

In 1956 the trapping program was designed to show whether infections are acquired seasonally, and whether the infections appear to be distributed uniformly in different habitats. Intensive snap-trapping in 3 dissimilar study areas ((1)

TABLE II. *Relationship of incidence of* Capillaria hepatica *infections to density of* P. maniculatus *population.*

Year	A Number of *P. maniculatus* examined	B Percent of *P. maniculatus* infected	C Percent increase or decrease of infection from previous year	D Density expressed as *P. maniculatus*/ 10 traps/night	E Percent increase or decrease of *P. maniculatus* population from previous year
1952	53	3.8	—	0.7	—
1953	261	7.7	+203	1.8	+257
1954	392	12.5	+162	2.8	+155
1955	63	3.2	−390	0.8	−350

maple-basswood hardwood slope, (2) mixed conifer-deciduous with some slash, and (3) aspen with hazelnut) was undertaken during the first week of each month May through September. Usually 200 traps were set out for 6 or 7 nights in each area during each trapping period. In addition, all other small mammals, which were collected from other study areas by various people, were examined. The results of these examinations are presented in table III.

The *P. maniculatus* were designated as juvenile, subadult, first adult, and second adult from the color of the pelage and the weight, following criteria used by mammologists of the Department of Lands and Forests. The second adult is an animal which was born in 1955 or earlier, i.e., had over-wintered, and all other age classes were born in 1956. Other species of animals were not assigned to age categories.

The population of *P. maniculatus* proved to be at a slightly lower level in 1956 than the previous low in 1952 (Falls, unpublished data), so that the number of *P. maniculatus* which were trapped relative to the total effort was small. Nevertheless, a total of 150 of this species were examined, of which 115 came from the three study areas. Obviously *C. hepatica* infections are not uniformly distributed (table III). If age structure of the *P. maniculatus* population is ignored, there is a pronounced decline in incidence from May through September. Note, however, that in the maple-basswood the *P. maniculatus* which harbored *C. hepatica* were second adults, so that most, if not all of these animals, must have acquired the infection the previous winter or earlier. In fact, of the 150 *P. maniculatus* which were examined from all study areas in 1956, 40 were second adults and 17 were infected with *C. hepatica,* whereas only 3 of 110 of all other age categories of *P. maniculatus* had this parasite. Since these mice rarely live much over a year, and the majority live a shorter time (Howard, 1949), most, if not all, of the infections were acquired during the cold months. Only 1 of the 150 *P. maniculatus,* and it not in the maple-basswood, harbored metacestodes. On the other hand, 4 of 46 *C. gapperi* had metacestodes and 3 had *C. hepatica.* These data corroborate the earlier conclusion that carnivore feces cannot be important in disseminating *C. hepatica* among *P. maniculatus.* The data in table III would appear to contradict the previous conclusion that the incidence of *C. hepatica* is dependent on the density of the *P. maniculatus* population, since 13 percent of this species were infected during a year of low population density. This increased incidence is due mainly to the extensive trapping done in the maple-basswood area, where intensive trapping was not done in previous years, although the incidence in the other areas was somewhat higher than anticipated.

In late May 1957 the same areas of the maple-basswood and the mixed conifer-deciduous areas were retrapped. Six of 10 *P. maniculatus* from the former area

were second adults and all harbored *C. hepatica*. In the other area, 7 of 12 *P. maniculatus* were second adults, but none of these mice had *C. hepatica*. Since none of the *P. maniculatus* born in 1956 and trapped that year in the maple-basswood area carried *C. hepatica* as late as in September, whereas all 6 of the overwintering animals were so infected in 1957, then most if not all of these infections must have been transmitted during the cold months of the year. One of 8 *C. gapperi* from the conifer-deciduous area had *C. hepatica*. None of the 14 other rodents from these areas harbored either *C. hepatica* or metacestodes.

A short trapping period in the maple-basswood area during November 1957 produced 8 *C. gapperi* and 7 *P. maniculatus* of which 2 were second adults. Metacestodes occurred in 1 *C. gapperi*, but no *C. hepatica* was found in any of the mice. In May 1958 the entire trapping program was confined to the maple-basswood area, but only 6 second adult *P. maniculatus* and 2 other rodents were captured in 600 traps set out on 4 consecutive nights. Something drastic must have happened to the population of all rodents during the previous fall and winter, since such a low spring population had not been encountered in previous years. Whether this low population or some other factor accounted for the complete absence of *C. hepatica* infections from this area was not determined.

The data from Algonquin Park are in marked contrast to those collected by Pavlov in Russia (see Skrjabin et al, 1957). In 1952 and 1953 he trapped mice from July through September and found a striking rise in the incidence of *C. hepat-*

TABLE III. *Examination of rodents in Algonquin Park in 1956**

Habitat	Species	May			June			July			August			September		
		Number			Number			Number			Number			Number		
		Trapped	With *C. hepatica*	With Metacestodes	Trapped	With *C. hepatica*	With Metacestodes	Trapped	With *C. hepatica*	With Metacestodes	Trapped	With *C. hepatica*	With Metacestodes	Trapped	With *C. hepatica*	With Metacestodes
Maple-basswood	*P. maniculatus*	*8***	*1*	—	*6*	*3*	—	*5*	*2*	—	*4*	*3*	—	*1*	*1*	—
	C. gapperi	0	—	—	0	—	—	15	—	—	15	—	—	14	—	—
	All other rodents	—	—	—	1	—	1	—	—	—	—	—	—	4	—	—
	Total	3	—	—	3	—	—	17	—	—	8	—	—	13	—	—
Mixed conifer-deciduous	*P. maniculatus*	11	4	0	10	3	1	37	2	0	27	3	0	32	1	0
	C. gapperi	*5*	—	—	*2*	*1*	—	*0*	—	—	*0*	—	—	*0*	—	—
	All other rodents	0	—	—	0	—	—	6	—	—	15	1	—	9	—	—
	Total	4	—	—	—	—	—	2	—	—	—	—	—	1	—	—
Aspen-hazelnut	*P. maniculatus*	1	—	—	3	—	—	17	—	—	10	—	—	11	—	—
	C. gapperi	10	0	0	5	1	0	25	0	0	25	1	0	21	0	0
	All other rodents	*2*	*1*	—	*0*	—	—	*0*	—	—	*0*	—	—	*0*	—	—
	Total	0	—	—	2	—	—	4	—	—	1	—	—	1	—	—
From all other sources	*P. maniculatus*	2	—	1	0	—	—	—	—	—	3	—	—	—	—	—
	C. gapperi	0	—	—	5	—	—	10	—	—	4	—	1	5	—	—
	All other rodents	4	1	1	7	0	0	14	0	0	8	0	1	6	0	0
	Total	*0*	—	—	*1*	*1*	—	*3*	*1*	—	*3*	—	—	*0*	—	—
	P. maniculatus	0	—	—	0	—	—	6	2	—	18	—	1	*4*	—	—
	C. gapperi	1	—	—	11	2	1	9	—	1	8	—	1	1	—	—
	All other rodents	2	—	—	7	—	—	24	—	—	20	—	—	9	—	1
	Total	3	0	0	19	3	1	42	3	1	49	0	2	14	0	1
	Grand total	28	5	1	41	7	2	118	5	1	109	4	3	73	1	1

* Less chipmunks.
** Italicized numbers represent second adults (overwintering).

ica in the young of the year at the beginning of September. Pavlov concluded that variations in the incidence of this parasite, among the 3 species of rodents which were infected, were due partly to the kinds of foods eaten and partly to where they lived since mice living in a flooded biotope had a higher incidence than those in a dry biotope.

THE LIFE HISTORY UNDER LABORATORY CONDITIONS

Various studies, particularly on the biology of the egg, were done in the laboratory. It was desirable to determine more about the embryonation of the egg under various conditions of temperature, and whether these eggs show any host specificity. In addition, we wanted to determine if the eggs somehow managed to pass out in the host's feces as do those of most other parasites living in the alimentary canal or its diverticula.

Cross-Infection Experiments

Eggs, originally isolated from natural infections in *P. maniculatus,* were fed to a variety of animals and infected 2 *Clethrionomys gapperi,* 38 of 46 *Mus musculus,* 1 *Ondatra zibethicus,* 7 *Peromyscus maniculatus,* 14 *P. leucopus* (new host record) and 1 *Tamiasciurus hudsonicus.* Three *Tamias striatus* and 2 *Cavia porcellus* were negative.

Migrating larvae were sought at daily intervals in blood and tissues of the liver and lungs, as well as in the wall of the gut in various hosts. Larvae were found in the liver as early as 2 days post-feeding. By the 12th to 13th day post-feeding the surface of the liver was knurled or showed distinct spotting, and from this point on an infection could be detected grossly. Three weeks after infection the liver lesions were pronounced, and in press preparations eggs were seen within some of the female worms, and a few eggs occurred in the liver. Within another month most of the adult worms were dead. Occasionally, in heavy infections, some worms occurred in the body cavity, and some of these still survived when those in the liver were overcome. This indicates that infections of about 2 weeks' duration could be detected in gross, routine examinations such as were done at Algonquin Park.

The Egg

The typical 2-plugged eggs ranged from 51 by 30 microns to 60 by 35 microns or 62 by 29 microns, and had an outer shell from 1 to 3 microns in thickness about halfway between the plugs. Occasionally larger, atypical eggs up to 69 by 35 microns in dimensions with an unusually thick outer shell were found. The abnormality encountered most frequently, both in the field as well as in the laboratory, showed an egg without an outer shell and containing a diffuse or granulated embryo. Occasionally such eggs were the only ones encountered when only 1 or 2 small foci of infection were present in the liver, which suggests such eggs may be infertile.

The embryo was in the 1-, 2-, or even 4-celled stage when the eggs were removed from the liver. If kept at 30° C most eggs were fully embryonated and infective in approximately 4 weeks, whereas at room temperature (ca. 23° C) 7 weeks or more were required for complete development. The vermiform embryo was active shortly after it was fully formed, but became quiescent within several weeks.

Luttermoser (1938) found that *C. hepatica* eggs from rats in Baltimore were killed after 2 weeks exposure at near 0° C. Pavlov (see Skrjabin et al, 1957) be-

lieved that eggs which were released in the autumn in Russia could not embryonate before cold weather killed them. Since winter temperatures are rather severe in Algonquin Park, and in view of transmission during the colder months, it was desirable to know more about the effect of lowered temperatures and prolonged storage on both embryonated and unembryonated eggs. Unembryonated and embryonated eggs kept for 60 days at – 15 to – 16° C developed infective embryos. However, unembryonated eggs did not withstand 22 hours at – 40° C. Fully embryonated eggs which were kept for 14 months at room temperature in 0.5 percent formalin still produced light infections, whereas other embryonated eggs stored for 2 full years under water at room temperature were no longer infective.

Egg Release from the Liver

Parasites which live in the alimentary canal, or its diverticula like the liver and lungs, commonly transfer to a new host via the feces of the infected host. Various workers have stated that few, if any, of the eggs of *C. hepatica* reach the intestine of an infected host. However, since *P. maniculatus* frequently suffer starvation during severe winters, and since starvation adversely affects the liver, conceivably a dietary deficiency might increase the number of eggs which reach the intestine. Accordingly, a series of experiments were arranged whereby eggs of *C. hepatica* were sought in the feces of mice kept on various diets.

In order to determine the number of eggs which were passed in the feces of rodents kept on a full diet fed ad libitum, a total of 20 fecal samples from *M. musculus,* 33 from *P. leucopus,* and 3 from *Tamiasciurus hudsonicus* were examined. All feces were from animals which were originally infected 3 to 5 weeks earlier. Only 1 egg was found from *M. musculus* and 10 eggs from *P. leucopus.* These observations corroborate those of previous workers who likewise found only a few eggs passed in the feces of an infected host.

A series of 7 experiments were undertaken utilizing 5 *M. musculus* in 1 experiment, 4 *P. maniculatus* in another, and a total of 15 *P. leucopus* in the remaining 5. The total food intake was limited in various ways for each animal in the first 6 experiments, and in the 7th mice were fed a diet deficient in protein. Eggs of *C. hepatica* were sought, following the procedures outlined above, from fecal concentrates, and when each mouse died or was killed, from the intestinal contents. The *M. musculus* had been infected nearly a year before experimentation began, but remaining mice had infections which were 2 to 7 weeks old. Five *P. leucopus,* which were exposed to infections comparable to the latter group, served as controls.

No eggs were found in the feces or intestinal contents of any *M. musculus.* Likewise, no eggs were found in the feces of *P. maniculatus,* although 2 had 4 and 5 eggs, respectively, in the total intestinal contents, and 1 had approximately 13 eggs per drop of sediment from the intestinal contents. One *P. leucopus* had 2 *C. hepatica* eggs in its feces on the 27th day of infection. Seven of the *P. leucopus* contained eggs in the intestinal contents, but 45 eggs were the most recovered from an animal.

Feces from the 5 *P. leucopus* kept as controls were examined beginning on the 21st day of infection. One mouse died with a 35-day-old infection. Eight eggs were recovered from the feces passed the day before death, and at autopsy several thousand eggs were recovered from the intestinal contents. This animal had a massive infection with many worms free in the body cavity. Eggs were not recovered from the feces of the other 4 controls, nor were there any eggs in the intestinal

contents when the mice were killed between 31 and 91 days after the original infection.

From these experiments it is evident that starvation due to restriction of total food intake or the maintenance of mice on a protein deficient diet does not induce an appreciable increase in the number of eggs pased in the feces. It is clear that early in the infection a few eggs may escape into the intestine, presumably via the bile duct. This is particularly apparent preceding "natural" death, or when the death process is prolonged, be it in a starved or well-fed mouse. This release did not occur if death was sudden, as when mice were killed with a sharp blow on the head, or in infections which were over 7 weeks old.

Since carnivores apparently are not important in releasing the eggs of *C. hepatica* from the liver of the mouse, and since starvation does not induce a significant increase in the release of eggs from the living mouse, then cannibalism is the remaining method most likely to do so. Eggs become so well encapsulated in connective tissue within 7 or 8 weeks after infection is acquired that digestion of the liver, which occurs when an animal is eaten, is one of the few ways eggs from such infections can escape. Such encapsulated eggs remain viable for a considerable time, since 37 percent of the eggs, removed from a *M. musculus* which had been infected 22 months previously, embryonated and were infective.

Two separate attempts were made to transmit *C. hepatica* in the laboratory under conditions which might be encountered in nature. In each trial both *M. musculus* and *P. maniculatus* were used in separate cages. The object of the first experiment was to determine if known infected mice passed eggs into the cage by defecation. In the second experiment infected livers were fed to both species of mice kept in other cages. After a suitable time, all mice were removed from their cages, and each cage remained fallow for 8 weeks. New mice were introduced into each cage, and kept there for 6 to 9 weeks, then all mice were autopsied. The contents of each cage were searched for eggs of *C. hepatica* on termination of each experiment.

None of the mice introduced into these cages became infected, nor did any of the mice which ate infected livers. No eggs of *C. hepatica* were recovered from the cages in which only infected mice had been kept. Eggs of *C. hepatica* were readily recovered from the contents of the cages in which mice had eaten infected livers, although none of these eggs had embryonated presumably on account of lack of moisture.

Lubinsky (1956) suggested, since human food is so readily contaminated with feces of rodents, that human infections with *C. hepatica* may be more common than is presently reported, particularly among the Canadian aborigines. Luttermoser (1938) and the present study showed that the environmental requirements, especially for moisture, are critical before eggs of *C. hepatica* will embryonate. Only under unusual conditions are rodent feces likely to fall into a suitable location where these eggs will both embryonate and become available to man. Further, the chances of exposure are even more remote when one considers that only the feces of a rodent or other animal, which has eaten an infected liver, harbors the eggs.

TRANSMISSION OF C. HEPATICA IN NATURE

It is now possible to postulate a cogent picture incorporating what is known about the biologies of *C. hepatica* and *P. maniculatus*. Howard (1949) found that

P. m. bairdii in Michigan leave the winter nest in the spring and establish a summer maternal nest for breeding activities. In the fall as many as a dozen or more mice band together into a larger winter nest, and in Howard's studies utilized the same experimental nest boxes during successive years. We suggest that the winter nest is the primary source of infection with *C. hepatica* in the population of *P. m. gracilis* which we studied. Probably most eggs are released in late winter when food reserves are gone and when starvation and cannibalism are most intense. Presumably the mice spend a great deal of time in the winter nest which will concentrate the nematode eggs. There is some question whether eggs released during one winter would become infective before the following spring. The very low incidence of infection in the fall in contrast to the high incidence in the spring indicates that either the source of infection is already present or that embryonation must occur during the winter. Certainly the eggs could survive in the nest since they can withstand temperatures of at least – 15° C for 60 days and still embryonate. The humidity within such nests probably is near saturation. As starvation continues coprophagy, and thus exposure to infection, may increase. The density of the population may determine the number of mice in the winter nest, and thereby affect the incidence of infection. The eggs within the winter nest remain to infect all animals which may partake of them during the warmer months.

SUMMARY

Capillaria hepatica is a common parasite in *Peromyscus maniculatus* in Algonquin Park, Canada, and occurs also in *Ondatra zibethicus, Clethrionomys gapperi, Microtus pennsylvanicus, Napaeozapus insignis,* and *Synaptomys cooperi,* the latter four being new host records. Laboratory infections were established in *Mus musculus, P. leucopus,* and *Tamiasciurus hudsonicus,* the latter two being new host records; *Tamias striatus* and *Cavia porcellus* were resistant to infection. The incidence of *C. hepatica* in a wild population of *P. maniculatus* varies directly with the density of the host. It was shown that predation could not account for the pattern of infection with *C. hepatica* in this population. Few eggs were released into the feces of the infected host, and these only early in the infection; starvation did not increase the number released. The eggs must be released from an infected host by cannibalism. Most infections in the population of *P. maniculatus* are acquired during the winter months, and it is postulated that the winter nest is the primary focus of infection. Eggs of *C. hepatica* are long-lived, both when embryonated and unembryonated, and since they survived temperatures of – 15° C, it is likely that they can withstand winter temperatures in Algonquin Park, at least within the winter nest.

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THE EFFECT OF IRRADIATION ON REPRODUCTION BY THE HETEROGENETIC GENERATION OF *STRONGYLOIDES PAPILLOSUS*. I. IRRADIATION OF MALES AND FEMALES*

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A relatively minute part of the literature on radiobiology is concerned with animal parasites and problems of direct parasitological interest. Such investigations have been carried out primarily on two common parasitic nematodes, *Ascaris* and *Trichinella*. The former has been used to a great extent in cytological and embryological studies dealing with cleavage and embryonic development (Bachofer, 1956; Zuppinger, 1928; and others). *Trichinella*, on the other hand, has been studied chiefly from the public health aspects where irradiation might serve as a means of sterilizing infected meat (Gould, et al, 1954) and in producing only intestinal infections as a means of initiating an immune response in the host (Levin and Evans, 1942). Recently, Villella, et al (1958) reported irradiation studies on *Ascaris* eggs as a possible basis for controlling this parasite of man.

Radiobiological techniques as employed in experimental embryology suggested an approach toward elucidation of certain factors involved in the unusual, if not unique, life cycle pattern in the nematode genus *Strongyloides*. *Strongyloides papillosus* and *S. ratti* differ from some other known species of *Strongyloides* in that they appear to have only one free-living bisexual generation. The full range of specific variability in the genus is, of course, not known. In view of the triploidy which has been observed in *S. papillosus* (Chang, 1953; Chang and Graham, 1957), the induction of successive free-living generations should be theoretically possible. It was, therefore, proposed to subject pre-copulatory free-living males and females to irradiation so that when cultured in vitro with non-exposed worms, reproduction might possibly occur by androgenesis or gynogenesis. Since Nigon and Roman (1952) reported pseudogamy in *S. ratti*, this approach appeared to be logical and important.

MATERIALS AND METHODS

Strongyloides papillosus, obtained originally from sheep at the School of Veterinary Medicine, University of Pennsylvania, was maintained in the laboratory in rabbits. Fecal pellets were collected when desired and cultured, usually intact, on wet filter paper in Petri dishes. The fecal worms were isolated by means of a Baermann apparatus.

Isolated nematodes were cultured in U. S. Bureau of Plant Industry Model watch glasses or depression slides, the concavities of which were filled with a nutrient agar-fecal extract medium diluted one-half with distilled water and adjusted to pH 7. Although the medium was sterilized after preparation, the nematodes were cultured under non-sterile conditions.

Mature or immature males and immature females were exposed to the radiation source and placed in cultures with unexposed worms of the opposite sex. Females alone were also cultured

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as controls for the virginity of the females of the bisexual cultures. The number of worms used as an inoculum depended upon the experiment. Ordinarily, a few of each sex were used to insure at least one copulating pair.

All cultures were incubated in the dark at 25 or 27 C. The artificial medium cultures were kept in moist chambers made from 90 by 15 mm or 150 by 20 mm Petri dishes with a piece of wet filter paper in the bottom section. Gallon ice cream containers were very useful in carrying and storing the large dishes. These were used in an inverted position so that when the bottom section was removed, the dishes would be neatly stacked in the low-bordered top section.

It was found expedient to use cobalt 60 gamma irradiation rather than X-rays. The high radiation sources available at the Brookhaven National Laboratory (BNL) reduced the time of exposure greatly and, bearing in mind the relative rapidity of development and the desire to return the worms to a "normal" environment as soon as possible, it was felt wise to do this. Furthermore, the procedures involved are simpler than with X-rays and, most important, the measurement of the dosage is more accurate even though there may be an error of 10 percent.

The isolated nematodes were placed in 13 by 99 mm "Lusteroid" tubes with 1 or 2 ml of aqueous fecal extract. Each tube was sealed with a rubber stopper. The tubes to be irradiated were placed in an aluminum carrier and lowered into the source of irradiation. Each tube was exposed only once and the worms of that tube were used only for that level of irradiation.

Although it would have been desirable to have always used the same radiation source so that all material would have been exposed at the same rate, this could not be done. Therefore, various isotopic cobalt sources were used, whichever was available at the time an exposure was desired. The sources used for this study had rates of irradiation ranging from 179 to 505 kiloroentgens (kr) per hour. It should also be noted that the rate of a particular source was subject to some variation. The dosimetry of the sources at BNL are measured about every 2 months and within this period of time there may be a change of about 2 percent.

The control tubes were shaken by hand to simulate the handling of the exposed tubes. Because of the handling, the worms were not separated prior to the time of exposure but afterwards, when ready to be cultured.

RESULTS

1. *Single Experiments*

A number of experiments involved the exposure of worms to different levels of irradiation on the same day or to the same dosage on different days. On each day of exposure, a single group of cultures of non-irradiated nematodes served as the controls. The results of these individual experiments have been combined and are recorded in tables I and II.

The data which accumulated were evaluated on the basis of (1) the average number of active larvae per bisexual pair of worms of the total number of pairs placed in culture $\left(\text{LPT} = \frac{\text{TL}}{\text{TP}}\right)$, and (2) the average number of active larvae per bisexual pair in those cultures positive for larvae $\left(\text{LPC} = \frac{\text{TL}}{\text{PP}}\right)$.

A. Irradiated males. The average number of larvae per pair of worms was, in general, low and varied from one experiment to the next for both irradiated and control organisms (table I; fig. 1). As would be expected, the LPC values are to a certain extent higher than the LPT values. The overall averages for the controls of these 2 sets of data as recorded in table I are 3.14 (LPT) and 3.78 (LPC).

Neither larvae nor eggs were found in 1 of the 3 experiments of the 5 kr group and 1 of the 2 experiments of the 10 kr group. However, all of the control experiments, but not all of the control cultures, were positive for larvae.

Two experiments constitute the observations at 15 kr. In one, a total of 19 active larvae were observed in 2 watchglass cultures, each containing 4 pairs of bisexuals. In the second experiment, 4 out of 10 cultures became positive and

Table I. The effects of irradiation on the production of S. papillosus larvae in single experiment cultures of irradiated males and non-irradiated females.

GROUP	NO. OF EXPTS.	CULTURES: TOTAL NO.	CULTURES: POS. FOR LARVAE	CULTURES: AGE, HRS.*	BISEXUAL PAIRS: TOTAL (TP)	BISEXUAL PAIRS: POS. FOR LARVAE (PP)	NUMBER OF ACTIVE LARVAE: RANGE IN CULTURES	NUMBER OF ACTIVE LARVAE: TOTAL (TL)	LPT§	LPC¶
5 Kr	3	35	11	42-96	44	17	1-30	105	2.39	6.18
Control	3	36	12	42-96	48	19	1-16	89	1.85	4.68
6 Kr	2	8	8	44	32	32	1-28	72	2.25	2.25
Control	2	8	7	44	32	28	1-15	31	0.969	1.11
8 Kr	2	8	5	96	32	20	1-6	15	0.469	0.750
Control	2	8	8	96	32	32	1-7	39	1.22	1.22
10 Kr	2	11	3	42-96	23	12	1-14	29	1.26	2.42
Control	2	11	5	42-96	23	14	1-10	17	0.739	1.21
15 Kr	2	12	6	96	18	12	1-15	24	1.33	2.00
Control	2	12	10	96	18	16	1-50	137	7.61	8.56
20 Kr	3	8	6	24-48	26	20	1-3	10	0.385	0.500
Control	3	8	7	24-48	26	20	9-38	127	4.88	6.35
35 Kr	1	4	0	72	16	0	-	0	0	0
Control	1	4	4	72	16	16	3-10	26	1.62	1.62
36 Kr	2	6	0	72-96	24	0	-	0	0	0
38 Kr	2	6	0	72-96	24	0	-	0	0	0
Control	2	6	4	72-96	24	16	1-11	21	0.875	1.31
40 Kr	5	19	3	45-144	70	12	1-4	6	0.0857	0.500
Control	5	18	16	45-144	66	36	1-55	273	4.14	7.58
42 Kr	1	4	1	48	16	4	-	1	0.0625	0.250
44 Kr	1	4	0	48	16	0	-	0	0	0
46 Kr	1	4	0	48	16	0	-	0	0	0
Control	1	4	4	48	16	16	16-22	78	4.87	4.87
50 Kr	1	6	0	48	24	0	-	0	0	0
Control	1	5	5	48	20	20	11-55	128	6.40	6.40
80 Kr	1	4	0	96	16	0	-	0	0	0
120 Kr	1	4	0	96	16	0	-	0	0	0
Control	1	4	4	96	16	16	1-17	36	2.25	2.25

*Maximum time of observation for active larvae. § LPT = TL/TP ¶ LPC = TL/PP

Table II. The effects of irradiation on the production of S. papillosus larvae in single experiment cultures of irradiated females and non-irradiated males.

GROUP	NO. OF EXPTS.	CULTURES: TOTAL NO.	CULTURES: POS. FOR LARVAE	CULTURES: AGE, HRS.*	BISEXUAL PAIRS: TOTAL (TP)	BISEXUAL PAIRS: POS. FOR LARVAE (PP)	NUMBER OF ACTIVE LARVAE: RANGE IN CULTURES	NUMBER OF ACTIVE LARVAE: TOTAL (TL)	LPT§	LPC¶
5 Kr	1	4	3	96	16	12	1-28	30	1.87	2.50
10 Kr	1	2	2	96	8	8	6-8	12	1.50	1.50
Control	1	4	2	96	16	8	1-3	4	0.250	0.500
15 Kr	1	2	2	96	8	8	50-55	105	13.1	13.1
Control	1	2	2	96	8	8	30-50	80	10.0	10.0
20 Kr	3	8	8	45-96	26	26	4-20	111	4.27	4.27
Control	3	8	7	45-96	26	24	12-50	198	7.61	8.25
35 Kr	1	4	4	72	16	16	1-14	35	2.19	2.19
Control	1	4	4	72	16	16	3-10	26	1.62	1.62
40 Kr	3	10	7	42-45	34	28	1-22	50	1.47	1.78
Control	3	12	11	42-45	42	40	2-60	252	6.00	6.30
50 Kr	1	4	2	48	16	8	-	2	0.125	0.250
Control	1	5	5	48	20	20	11-55	128	6.40	6.40
80 Kr	1	2	0	96	8	0	-	0	0	0
120 Kr	1	2	0	96	8	0	-	0	0	0
Control	1	4	4	96	16	16	1-17	36	2.25	2.25

*Maximum time of observation for active larvae. § LPT = TL/TP ¶ LPC = TL/PP

yielded 5 active and at least 3 dead larvae at 96 hours. Eighty and 57 active larvae were scored for the controls of these 2 experiments.

In one experiment of the 20 kr group, only 1 larva was found in 1 of 2 cultures. Although eggs were found in the negative culture, no larvae were observed by 96 hours. The remaining 2 tests at this dose level were made up of 3 cultures each;

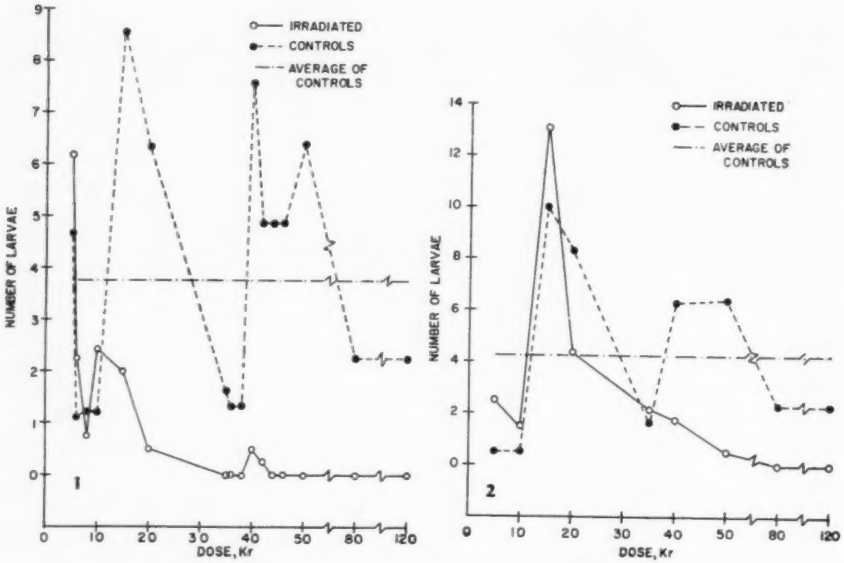

FIGURE 1. Average number of active larvae per pair of irradiated male and non-irradiated female *S. papillosus* in cultures of single experiments which were positive for larvae (LPC).

FIGURE 2. Average number of active larvae per pair of irradiated female and non-irradiated male *S. papillosus* in those cultures of single experiments which were positive for larvae (LPC).

4 pairs per culture produced a total of 3 active larvae in 1 experiment, while in the other experiment 2 pairs per culture produced 5 larvae.

Neither eggs nor larvae were found in cultures of worms exposed to 35 kr. Eggs were found in 1 culture of the 36 and in 1 culture of the 38 kr group at approximately 24 hours. Active adults were observed in both groups at this time. However, subsequent examinations until 96 hours of incubation failed to reveal larvae. Eggs were found in all control cultures, and all of these cultures but 1 were positive for larvae by 96 hours.

Two of the individual experiments making up the 40 kr group were positive for larvae. Of the 19 cultures made for this level, 9 occurring in 3 experiments were positive for eggs; in contrast to this, 17 of 18 control cultures produced eggs. Survival of adults varied but was similar for both the irradiated and controls. The single larva found in 1 of the 3 cultures of the first experiment at this dose level developed to the filariform larval stage and was still active at 116 hours. In the second experiment at 40 kr, 5 of 7 cultures had eggs at 24 hours. However, by 144 hours of incubation, only 5 larvae were recovered from 2 of these cultures and these two had developed to the filariform stage.

In the 42 kr group, the culture which yielded the single larva was the only 1 of 4 to have eggs deposited in the medium. Although neither eggs nor larvae were found in cultures of nematodes exposed to 44 and 46 kr, control cultures yielded 78 larvae.

Five of the 6 cultures of the 50 kr group became positive for eggs by 48 hours. Although developing larvae were observed in some at 24 hours, none hatched.

Three of the cultures of the 80 kr group and 1 of the cultures of the 120 kr group had eggs at approximately 24 hours; after 96 hours of incubation none had yielded larvae.

B. Irradiated females. Except for the results at the 5 kr level, more larvae were produced in bisexual cultures with irradiated females than in cultures with irradiated males. As in the case of the irradiated males, the results varied from one experiment to the next (table II, fig. 2). The averages of all the control LPT and LPC values, as listed in table II, are 4.07 and 4.23, respectively.

The 3 experiments making up the 20 kr group consisted of 2, 3, and 3 cultures with 4, 4, and 2 pairs of adults in each, respectively. These produced 35, 36, and 40 active larvae, while the corresponding control cultures yielded 80, 65, and 53 active larvae.

Only 2 of the 3 experiments at the 40 kr level produced larvae. Out of 3 and 4 cultures, each with 4 pairs of worms, 17 and 22 active larvae were observed. The third test was made up of 3 cultures with 2 pairs of bisexuals in each. Two of these cultures were positive for eggs at 48 hours. At that time, the eggs appeared to be undergoing cleavage. However, at 96 hours, no larvae were present.

In the 50 kr group, all cultures had eggs deposited in the medium but only 2 produced larvae. One of the 2 larvae developed into the filariform stage and was active at 120 hours. One culture had embryonating eggs, but none had hatched by 48 hours.

One culture of the 80 kr and 1 culture of the 120 kr groups had eggs deposited in the medium but larvae were never observed.

2. Compound Experiments

Samples of worms from the same coprocultures were irradiated at different doses in the same source, a single set of control cultures serving for all the exposed groups. These experiments provided a clearer picture of the effects of irradiation on *Strongyloides papillosus* than the single experiments recorded above.

A. Irradiated males. The lowest dose to which the males of this series were subjected was 20 kr. Larval production was observed at this dose but not at higher doses (table III). The total number of active larvae from 20 kr exposure was 3

Table III. The effects of irradiation on the production and hatching of eggs (counted at 44 hours) and survival of the larvae (counted at 66 hours) of S. papillosus in cultures of a compound experiment.

DOSE, Kr	TYPE*	CULTURES NO.	NO. EGG POS.	NO. LARVA POS.	BISEX. PAIRS TP #	PP @	EGGS NO.	EGGS PERCENT HATCHED	NUMBER OF LARVAE ACTIVE (TL)	ACTIVE + DEAD	LPT §	LPC ¶	INDUCED DOMINANT LETHALS
20	XM+CF	4	3	3	8	6	64	9.37	3	6	0.375	0.500	0.881
	XF+CM	4	2	2	8	4	36	100	31	37	3.88	7.75	-0.261
40	XM+CF	4	2	0	8	0	8	0	0	0	0	0	1.00
	XF+CM	4	1	0	8	0	6	0	0	0	0	0	1.00
60	XM+CF	4	2	0	8	0	16	0	0	0	0	0	1.00
	XF+CM	4	1	0	8	0	3	0	0	0	0	0	1.00
80	XM+CF	4	1	0	8	0	2	0	0	0	0	0	1.00
	XF+CM	4	1	0	8	0	14	0	0	0	0	0	1.00
100	XM+CF	4	0	0	8	0	0	-	-	-	-	-	-
	XF+CM	4	0	0	8	0	0	-	-	-	-	-	-
	CM+CF	8	5	5	16	10	111	79.3	82	88	5.12	8.20	-

*XM+CF = irradiated males with non-irradiated females.
XF+CM = irradiated females with non-irradiated males.
#Total number. @Number positive for larvae. §LPT = TL/TP. ¶LPC = TL/PP.

larvae after 66 hours of incubation. At least 1 of these larvae developed to the filariform stage. Although 3 males and 2 females were placed in each culture, a total of 8 pairs of worms are considered to have been present, and, therefore, the LPT value is 0.375 and the LPC value is 0.500. If the number of dead larvae are considered, these figures are 3.88 and 7.75, respectively.

The percentages of eggs not hatching in cultures of irradiated worms was 90.6 at 20 kr while in the controls it was 20.7. At 40, 60, and 80 kr, 100 percent of the eggs laid did not hatch; at 100 kr, no eggs were noted.

From this type of experiment, the induced rate of dominant lethals produced by irradation could be calculated using the formula $a - b/1 - b$, where a is the frequency of eggs not hatching in the cultures with irradiated worms, b is the frequency of the control eggs that do not hatch, and $1 - b$ is the frequency of the control eggs that do hatch. As can be noted in table III, positive effects occurred at all levels to which the males were exposed.

B. Irradiated females. Larvae were found in cultures of the 20 kr samples as in the case of cultures with irradiated males. The total number of active larvae produced in cultures with irradiated females (20 kr) was over 10 times greater than in cultures with irradiated males (31 to 3). The LPT and LPC values indicate that there was not a great difference between the cultures with females exposed to 20 kr and control cultures.

The percentage of hatching at 20 kr was 100 percent due to the fact that 36 eggs were counted at 44 hours and 37 larvae, active and dead, were counted at 66 hours. The percentage of non-hatching is, therefore, taken to be 0 for the 20 kr cultures, and 100 percent for the cultures of the other groups compared to 20.7 percent for the controls. No eggs were found in the 100 kr samples.

The induced rate of dominant lethals was found to be a negative value (−0.261) for the 20 kr group and positive for the other groups where eggs were found in the medium.

DISCUSSION

The efforts to attain the objectives outlined above have resulted in this study of immediate irradiation effects on reproduction in *S. papillosus*. Papers by Galliard (1951) and Katz (1956) are, apparently, the only reports on the effects of irradiation on species of *Strongyloides*. Galliard's (1951) work was concerned with the effects of irradiation on *S. stercoralis* and involved exposing infected dogs to X-rays. The report by Katz (1956) dealt with parts of the subject matter presented in this paper.

In reviewing the literature on the irradiation of nematodes, one finds that 20 and 40 kr has been a frequently used dosage. Although it may be just a matter of chance or convenience that these levels were employed, the results reveal an interesting if not important common observation concerning the effects of 20 and 40 kr irradiation on nematode reproduction and development.

Babero (1952, p. 118) states the following:

> The morphological abnormalities which resulted from the exposure of fertile *Ascaridia galli* eggs to 20,000 and 40,000 roentgen units were more varied and more pronounced than those abnormalities found in larvae which developed from eggs which were exposed to 5,000 and 10,000 roentgen units.

Babero (1952) also concluded that 20 and 40 kr adversely affected the development of these eggs.

Working with *Rhabditis strongyloides,* Thomas and Quastler (1950) observed that 20 and 40 kr brought about changes in the rhabditin granules of the intestine and that 40 kr produced morphologic alterations in the reproductive systems of males and females and in eggs and larvae. Changes in subsequent generations were noted and, therefore, it was concluded that these levels of irradiation affected the genetic components of the organisms. It has recently been reported by Ciordia (1957) that infective *Trichostrongylus axei* larvae exposed to 20 kr did not mature after being fed to rabbits. However, larvae exposed to 10 kr were not affected.

In the case of the single experiments (figs. 1 and 2), the average number of active larvae per pair of worms with either irradiated male or irradiated female dropped after 15 kr. Even though a LPC value less than one occurred at 40 and 42 kr in the case of irradiated males, at 44 kr and higher doses no larvae were found. In cultures with irradiated females, the LPC value was less than one at 50 kr and no larvae were found at 80 and 120 kr.

Actually, during the period of experimental work, it had been noted that 20 and 40 kr were critical and this accounts for the fact that more irradiated series were made at these dosages than at others (see tables I and II). Thus, there is agreement with those who have observed these levels to be of importance in radiobiological studies with nematodes.

Compound experiments, utilizing several levels of exposure show much more clearly the linear effect of irradiation on *S. papillosus* larval production. Twenty kr was the lowest dose used and, since no larvae were produced at 40 kr, the effect appears to be immediate and marked. It would, therefore, be of great value to carry out such experiments at doses below 20 through 40 kr and higher at short increments. With large samples, it may be shown that some reproduction can actually take place above 40 kr as was observed in the single experiments.

It must be remembered that in this study 2 biological entities were involved: males in the 4th or 5th stage of development and females in the 3d or 4th stage. Basir (1950) has published a full description of the morphological features of *S. papillosus* and illustrations of the stages referred to here. Irradiation, therefore, could have affected various cells in different stages of development. In the male, various stages of maturation of spermatozoa would have been subjected to irradiation. The same would be true for the various stages of development of the genital organs and ova of the females. And, of course, at the time the germ cells were being irradiated, "whole body" irradiation of the worms was also occurring. However, it seems that the doses used did not prevent immature females from completing their development since eggs were found in cultures of females exposed to 80 kr (table III) and 120 kr (single experiments).

In irradiating male *S. papillosus* when spermatozoa were present, the effects on the progeny and reproduction itself would probably be more pronounced than when females were irradiated before oogenesis had really begun. This, then, may explain the higher larval scores for pairs with irradiated females than with irradiated males.

It has been well established that cells differ in their sensitivities to radiation. Cells undergoing division are believed to be more sensitive than resting cells. Rollason (1949), for example, has reported that in *Rana pipiens* ova undergoing maturation division were more sensitive to the effects of X-rays than resting cells, and

that fertilized eggs were more sensitive than uterine or ovarian eggs while the uterine eggs were more susceptible than the ovarian.

Observations have been made on parasites along these lines. Seide (1925) reported that the older the *Ascaris* embryos are at the time of irradiation, the more resistant they are to the effects of radiation when massive doses of roentgen and radium radiation are employed. However, if ultraviolet light is used, the older the embryos, the more susceptible are they to this form of irradiation. He concluded also that the eggs undergoing the first cleavage are highly susceptible to radium treatment when the chromatin occurred in the form of fully developed chromosomes.

More recently, Villella, et al (1958) found the embryonated eggs of *Ascaris lumbricoides suum* to be more susceptible to cobalt 60 than unsegmented eggs. Moreover, lower doses could be used with X-rays than cobalt 60 in order to obtain the same effect.

The value of interpreting the data of this study in more defined terms than larval production is of importance in radiobiological investigations, and, therefore, the "dominant lethal effect" has been applied to the present work. Such a condition can only be concluded from the results at hand since, where present, dominant lethals result in the death of the organism, and, therefore, cannot be transferred to progeny (Lea, 1955). The negative value obtained for irradiated females in the compound experiment (table III) indicates that (1) there may be no significant difference between the irradiated and control organisms and (2) no dominant lethals were probably produced. However, in the case of the irradiated males, it appears that this phenomenon did take place.

SUMMARY

Fourth and fifth stage male and third and fourth stage female *S. papillosus* were subjected to various doses of cobalt 60 gamma irradiation and cultured in vitro with controls of the opposite sex. Fewer offspring were produced in cultures containing irradiated males than in cultures of irradiated females.

A single experiment involving several doses and one control gave a more linear picture of the effects of irradiation than individual experiments carried out at various times for different dose levels. In the former, larvae were obtained in cultures of nematodes exposed to 20 kr but not 40, 60, 80 or 100 kr; in the latter, larvae occurred in cultures of males irradiated at 42 kr and in cultures of females exposed to 50 kr but not at the higher levels employed.

With increased doses of irradiation, there was a decrease in the percentage of eggs hatching.

The dominant lethal effect was observed in cultures with males exposed to 20 kr and higher doses. In cultures with irradiated females, this phenomenon was observed at 40 kr and higher levels.

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AGE RESISTANCE OF CATTLE TO NEMATODES OF THE GASTROINTESTINAL TRACT

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The literature is replete with references to a greater resistance in older animals than in younger ones against the establishment of nematode parasites. Experimental data confirming the existence of age resistance have been presented for a variety of nematode host systems: viz, *Ancylostoma braziliense* in cats and dogs (Sarles, 1929); *Nippostrongylus muris* in rats (Graham, 1932 and Chandler, 1932); *Strongyloides ratti* in rats (Sheldon, 1937); *Trichinella spiralis* in rats (Nolf and Zaiman, 1941); and *Ascaridia galli* in chickens (Ackert, Porter and Beach, 1935). On the other hand, there was no evidence of age resistance to *Trichostrongylus calcaratus* in rabbits (Sarles, 1932) or to *T. colubriformis* in guinea pigs (Herlich, 1958).

Although there has been considerable reference to age resistance against nematode parasites in ruminants, evidence of this phenomenon is largely empirical being based primarily on field observations of naturally infected cattle. Under these circumstances, varying degrees of acquired immunity cannot be ruled out as a factor in the causation of the observed facts. Experimental data have been presented by Lucker (1952) and Goldberg (1952) suggesting that older sheep are somewhat more resistant than younger ones to infection with *Haemonchus contortus* and *Oesophagostomum venulosum*. However, Gordon (1950) stated that "age resistance appears to be practically absent as far as haemonchosis is concerned" in sheep Furthermore, Stewart and Gordon (1953) reported that 7 of 10 three- to four-year-old sheep raised worm-free died from experimental exposure to *T. colubriformis*.

Host age appeared to have no influence on susceptibility to infection with *O. radiatum* and *Bunostomum phlebotomum* in calves ranging from 0.5 to 1.25 years old (Mayhew, 1940) or to *Cooperia punctata* in cattle up to 1 year old (Bailey, 1949). On the other hand, the course of infections of calves with *Strongyloides papillosus* was affected by age of the host (Vegors, 1954). In rather striking contrast, however, various investigators (Bailey and Herlich, 1953; Andrews et al, 1953; and Bailey and Thorson, 1954) have incriminated gastrointestinal nematodes as the cause of severe clinical parasitism and deaths in adult cattle.

As there is a paucity of unequivocal experimental evidence of age resistance to nematode parasites in cattle, some experiments were conducted in an attempt to clarify the status of this phenomenon; the results are herein reported. Some of the data from one of the experiments have been previously published in abstract form (Herlich, 1956b).

PROCEDURE

Four experiments were conducted using 13 calves from 5 to 8.5 months old and 18 steers from 18 to 25 months old. The cattle included grade Jerseys and Jersey-Guernsey and Jersey-Holstein crosses. All calves and 11 of the adults were raised free of all helminths other than

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Strongyloides papillosus, while the other 7 adults were raised on a so-called dry-lot where they acquired low-level infections of *Cooperia* sp., *Trichostrongylus* sp., and *Oesophagostomum* sp. The numbers of calves and adult cattle used in each experiment are shown in tables I and II.

In experiment 1, the cattle grazed together from February 20 to April 12, 1956, a total of 51 days on a pasture with a lush stand of winter oats. One-fifth of that pasture had been fenced off and artificially contaminated with cattle manure containing nematode eggs. The cattle spent a total of 4 weeks over 3 different intervals grazing the contaminated portion of the pasture; the balance of grazing time was spent on the uncontaminated portion.

TABLE I. *Weight performances and maximum fecal egg counts of naturally infected calves and adult steers, and numbers of worms recovered from them post mortem.*

No.	Animal Age (mos.)	Breed	Max. E.P.G.*	Net wt. change (lbs.)	Total no. worms	Immature worms (percent)
			Experiment 1			
1	5	J**	280	70	16,520	40
2	5	J	168	62	20,360	31
3	5	J	84	87	56,280	66
		Average	177	73	31,053	53
4	18	J	24	43	19,860	53
5	18	J	36	40	9,040	51
6	18	J	20	45	6,760	36
		Average	27	43	11,887	49
			Experiment 2			
7	6.5	J	102	50	31,364	3
8	6.5	J	1,886	−25	471,000	2
9	5.5	J	334	− 5	449,259	2
10	4	J	490	8	141,938	17
		Average	703	7	273,390	4
11	20.5	J-H***	26	20	2,185	2
12	20.5	J	46	10	198,133	71
13	20.5	J	32	20	9,211	5
(14)	20.5	J-H	8	17	23,901	46
		Average	28	17	58,358	65
			Experiment 3			
15	6.5	J	900	55	85,541	10
16	6	J-H	978	65	46,071	9
17	6	J	1,205	40	45,987	6
		Average	1,028	53	59,200	8
18	23.5	J-G****	80	60	36,457	9
19	23	J	136	35	20,091	17
(20)	23.5	J-H	30	60	119	0
(21)	23.5	J	136	30	27,614	11
(22)	23	J-H	136	60	38,609	21
		Average	104	49	24,578	15

* Eggs per gram of feces.
** Jersey.
*** Jersey-Holstein cross.
**** Jersey-Guernsey cross.
Animal No. in parentheses indicates adult with pre-experiment low-level nematode infection.

In experiment 2, the cattle grazed together from April 23 to June 10, 1957, a total of 47 days rotating over 3 pastures of mixed grasses, Dallisgrass predominating. These pastures were naturally and artificially contaminated.

In experiment 3, the cattle grazed together from July 21 to Sept. 9, 1958, a total of 49 days on the same pastures utilized in experiment 2. During the final 3 weeks of the experiment, the pastures were poor; consequently, a supplement of hay was fed. The pastures were rested during the interval between experiments 2 and 3, and no manure was added during that time.

In experiment 4, each test animal was given per os a single dose of 550,000 infective larvae of a mixture of species of gastrointestinal nematodes of cattle. Some of the larvae were supplied by Dr. F. W. Douvres, A.R.S., U.S.D.A., Beltsville, Maryland. All larvae were mixed together in a tube and then divided into 9 equal doses. The cattle were housed in a barn to preclude extraneous helminth infections.

In all experiments, the animals were weighed once a week at which time feces were collected. Feces were examined for nematode eggs by the DCF technic using zinc sulfate solution as the flotation fluid.

Hematological observations were made as follows: serum calcium and phosphorous levels by the method of Fiske and Subborow (1923), and total protein in serum by the biuret method (Kingsley, 1939) in experiments 2, 3, and 4; packed cell volumes and blood glucose levels (Somogyi, 1945) in experiments 3 and 4. In experiment 1, no blood studies were carried out.

TABLE II. *Weight performances and maximum fecal egg counts of artificially infected calves and adult steers, and numbers of worms recovered from them post mortem.*

No.	Animal Age (mos.)	Breed	Max. E.P.G.*	Net wt. change (lbs.)	Total no. worms	Immature worms (percent)
			Experiment 4			
23	8.5	J**	416	10	48,012	< 2
24	6	J-G***	1,340	40	115,555	< 2
25	6	J-H****	3,295	−10	104,855	< 2
		Average	1,684	13	89,474	< 2
26	25	J-G	470	20	21,444	< 2
27	25	J-H	390	20	18,345	< 2
28	25	J-H	116	25	10,768	< 2
		Average	325	22	16,852	< 2
(29)	25	J-H	64	30	14,218	< 2
(30)	25	J	410	25	20,428	< 2
(31)	25	J-G	54	30	10,757	< 2
		Average	176	28	15,134	< 2
Average for 6 adults			251	30	15,993	< 2

* Eggs per gram of feces.
** Jersey.
*** Jersey-Holstein cross.
**** Jersey-Guernsey cross.
† Animal No. in parentheses indicates adult with pre-experiment low level nematode infection.

All animals in experiments 1, 2, and 3 were killed at the end of the grazing period, whereas the artificially infected animals of experiment 4 were killed 43 days after larvae were administered. Worms were recovered and numbers determined by the methods described by Porter (1942) and Herlich (1956a).

RESULTS

Course of infection. During the third week of each experiment, nematode eggs appeared in the feces of the young and mature cattle that had been raised helminth-free. Simultaneously in experiments 2, 3, and 4, there was an increase in the number of eggs per gram of feces (EPG) from the steers that had pre-experiment low-level worm infections; however, the maximum EPG was essentially the same for the adult animals regardless of whether or not they were reared helminth-free (tables 1 and 2). In each experiment the average maximum EPG of the calves was greater than that of the adults, and individual maximum counts of the calves exceeded those of the mature steers, with one exception in experiment 4.

Calves 8, 9, and 10 in experiment 2 and calf 25 in experiment 4 developed clinical symptoms of parasitism. They scoured severely during the final 2 to 3 weeks of the experiments and became emaciated. Three of them lost weight (tables 1 and 2). The remaining animals were not visibly affected by their infections.

Hematological observations. There were no consistent or marked changes in packed cell volumes, or in levels of serum calcium and blood sugar. The levels of phosphorous and total protein fluctuated slightly and erratically in all animals except in those calves which were clinically affected. In these animals there were marked and constant reductions in the levels of serum phosphorous and total protein; terminal levels ranged from 33 to 68 percent and from 24 to 37 percent, respectively, below pre-infection levels.

Post-mortem observations. The numbers of worms recovered from each animal and the percentages of those numbers that were immature are shown in tables 1 and 2. Species present were: *Haemonchus placei, Ostertagia ostertagi, Trichostrongylus axei, T. colubriformis, Cooperia punctata, C. pectinata, Nematodirus helvetianus,* and *Oesophagostomum radiatum.* Predominant species in experiments 1 and 2 were *O. ostertagi* and *T. axei;* in experiment 3, *Cooperia* spp.; and in experiment 4, *O. ostertagi* and *Cooperia* spp. Within the different experiments, the

number of worms per animal varied considerably in each age group. As regards numbers of worms harbored, there was little difference between the adult bovines raised helminth-free and those that were not and no notable difference between the various breeds of cattle.

In each pasture experiment, the adult steer harboring the most worms had as many as or more worms than the calf harboring the fewest worms. However, in experiment 4, where the animals were artificially infected, the mature bovine with the most worms had less than half as many as the calf with the fewest worms. The juvenile group in each experiment averaged more worms of each species than the adult group or groups, with the exceptions of *H. placei* in experiment 1 and *O. radiatum* in experiments 2 and 3. The average number of those two species was small, 550 or less. Adult cattle appeared to be almost completely refractory to *N. helvetianus, C. pectinata,* and *T. colubriformis.*

No measurements were made of adult parasites; however, as the worms were picked from aliquots for population estimates, no noticeable size difference was observed between the worms recovered from the calves and the adult cattle.

Severe pathological alterations were noted in the abomasa of the clinically affected calves of experiment 2 and of adult bovine 12 of that same test. These stomachs were highly inflamed; discolorations ranged from deep red to purple. The stomachs and adjoining mesenteries were extremely edematous, and the abomasal mucosa was ulcerated and studded with myriad clusters of nodules. In general, the abomasa of all other animals were normal, as were the intestines of all animals except the clinically affected calf of experiment 4. In that animal, there were petechial hemorrhages in the first 3 to 4 inches of the duodenum, and the anterior 15 feet of the small intestine were hyperemic.

DISCUSSION

Resistance to nematode infections may be reflected by: (1) an increase in the length of the prepatent period; (2) stunting of mature worms; (3) reduced egg production; (4) inhibition of larval development; and (5) the number of worms established in the host. The phenomenon of resistance to the effects of infections also exists.

In these experiments, age of the host did not appear to have any effect on either the prepatent period of infection or the size of the mature worms. Also, within the limitations of the fecal egg count technic employed, age apparently did not influence nematode egg production.

Within each pasture experiment, the percentages of worms that were immature varied considerably from animal to animal, with the larvae representing a large proportion of the worm population in some juvenile and adult cattle. This may have been an indication of inhibited larval development due to an acquired resistance stimulated by repeated larval ingestion or merely of larval acquisition shortly before death of the host. However, the uniformly low percentages of immature worms recovered in experiment 4 where the animals were equally infected artificially by a single administration of larvae indicate that age, per se, does not affect rate of larval development.

There was, however, definite evidence of a greater resistance in the older animals against infection and the effects of infection. In experiments 1 to 3, the pasture

experiments, the calves had an average of about 2.5 to 4.5 times more worms than the adult cattle. Although these differences are not statistically significant, it should be noted that, on the basis of the relative ages and weights of the experimental animals, the mature cattle should have consumed about 3 times more forage daily, and presumably, therefore, would have ingested many more infective larvae than the calves. In experiment 4, which excluded differences in total larval acquisition, the calves had an average of slightly over 5.5 times more worms than the adult cattle, a difference that is significant at the 1 percent level. The results of these experiments suggest that there is in cattle an age resistance which may be modified by differences in total larval intake that may be related directly to the difference in amount of forage consumed, or to chance grazing of more or less heavily contaminated parts of the pasture. The latter possibility is indicated by the fact that at least one adult in each pasture experiment had as many as, or more worms than the calf with the least worms.

Of the 13 calves used, 4 were unmistakably ill and the severity of the effects of their infections was almost in direct proportion to the number of worms they harbored. On the other hand, none of the adult steers was visibly affected, despite the fact that steer 12 in experiment 2 had almost 200,000 worms post mortem, some 56,000 worms more than the number present in calf 10 which was one of the clinically affected animals. This suggests that age of the host influences the effects of the worms on the host as was further indicated by the absence of any hematological aberrations in adult steer 12.

SUMMARY

Four experiments were conducted to study the age resistance of cattle to nematodes of the gastrointestinal tract. The experiments used 31 cattle, including 13 calves from 5 to 8.5 months old and 18 steers from 18 to 25 months old.

In three experiments, 22 of the cattle were grazed on contaminated pasture and became naturally infected with some 8 species of gastrointestinal nematode parasites. In one experiment, 9 of the cattle were kept in a barn and artificially infected.

The results of these experiments indicated that by virtue of age, per se, the adult cattle were generally more resistant to nematode parasites and their debilitating effects than the calves. Under natural conditions, however, the individual adult animals did acquire appreciable numbers of nematode parasites.

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A NEW TAPEWORM, *CHOANOTAENIA ANGOLENSIS*, N. SP. FROM THE ANGOLA PITTA, *PITTA ANGOLENSIS*, IN SOUTHERN RHODESIA

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A single male specimen of the Angola Pitta, *Pitta angolensis*, was brought into the Department. On examination, several cestodes were found in the intestine, which appear to represent a new species in the genus *Choanotaenia* Railliet, 1896. The name *Choanotaenia angolensis* is proposed for this new worm.

All measurements are in millimeters unless stated otherwise.

Choanotaenia angolensis n. sp.

Description: Body small; maximum length 73, maximum width 0.62. Scolex diameter 0.35 to 0.39. Extruded rostellum 0.079 in diameter. Hooks 22, double row, 80 microns long. Rostellar sac up to 0.31 long. Four suckers, diameter 0.19 to 0.21, unarmed. Mature and gravid proglottids longer than wide. Typical mature segment 0.9 long by 0.54; typical gravid segment 1.59 long by 0.57. Testes 31 to 34 per proglottid, diameter 0.047 to 0.056, postovarian, confined within lateral excretory canals. Cirrus-sac running forward from genital atrium, 0.18 to 0.19 long by 0.042. Diameter extruded cirrus 9 to 11 microns; unarmed. Bunch of setae at base of cirrus. Internal vesicula seminalis, thin and coiled.

Genital atrium alternating irregularly, opening on lateral margin, in anterior third of proglottid. Vagina, 6 to 9 microns in diameter, opening into genital atrium posterior to cirrus. Receptaculum seminis large, pyriform, diameter 0.085 to 0.092. Ovary bilobed, aporal lobe being considerably the larger, lying between anterior and middle thirds of body.

Looped duct 11 microns diameter connecting receptaculum seminis with oviduct; lying dorsal to other genitalia. Oviduct ventral, running posteriad to Mehlis' gland, which is spherical, 47 to 50 microns diameter, overlying anterior border vitellarium. Latter 0.097 to 0.106 diameter, irregular, median, ventral and postovarian. Gravid uterus breaking down to form egg capsules each containing one egg. Capsule diameter 0.054 to 0.068; onchosphere diameter 0.035 to 0.038.

Host: Pitta angolensis Vieillot.

Location: Intestine.

Locality: Near Salisbury, Southern Rhodesia. Types to be deposited in the British Museum (Natural History), London.

DISCUSSION

The taxonomic position of the genus *Choanotaenia* Railliet, 1896, and its relationship to the genera, *Anomotaenia* Cohn, 1900, and *Paricterotaenia* Fuhrmann, 1932, have already been discussed at length (Mettrick, 1958). The position has not changed since then, and Lopez-Neyra's (1952) emended generic definition is not acceptable in our present state of knowledge. Fuhrmann's (1932), definition of the genus *Choanotaenia* states that there may be 1 or 2 rows of hooks, and that the uterus breaks down into egg capsules.

There are at present 14 species in the genus *Choanotaenia* which are recorded from passerine birds. Those with 2 rows of hooks are *C. fieldingi* (Maplestone and Southwell, 1925), *C. galbulae* (Gmelin, 1790), *C. musculosa* (Fuhrmann, 1896), *C. passerina* (Fuhrmann, 1907), *C. spinasocapite* Joyeux and Baer, 1955, and *C. sinensis* Joyeux and Baer, 1955. In the following species, the number of rows of hooks is unknown: *C. orioli* Joyeux and Baer, 1955; *C. platycephala* (Rudolphi, 1810); *C.*

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microsoma (Southwell, 1922); and *C. taylori* (Johnston, 1912). *Choanotaenia angolensis* n. sp. may be distinguished from all these species by using one or more of the following characters: number of hooks, size of hooks, number of testes, and size of the cirrus-sac.

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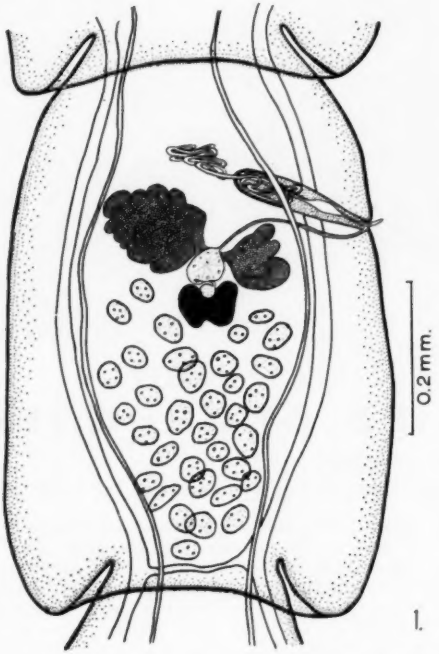


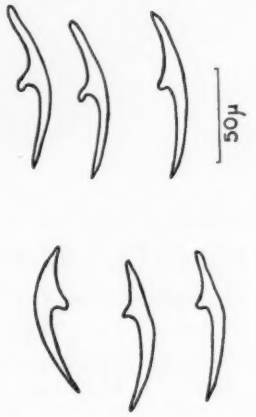
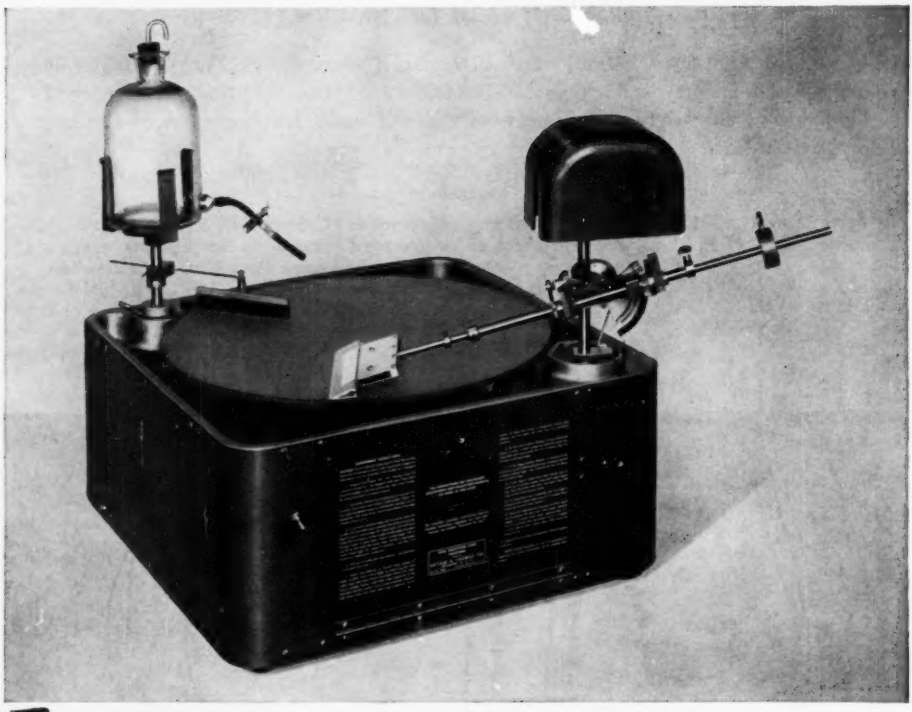


Figure 1. Mature segment of *Choanotaenia angolensis* n. sp.
Figure 2. Rostellar hooks of *Choanotaenia angolensis* n. sp.



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